



Article Multi-Gene Phylogeny and Taxonomy of *Hydnellum* (Bankeraceae, Basidiomycota) from China

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Abstract: The genus Hydnellum is an important group of stipitate hydnaceous fungi which can form ectomycorrhiza with many species of woody plants. In recent decades, the frequency and number of basidiocarps observed in China have been declining significantly. So far, however, we know little about the species diversity of Hydnellum in China. In this study, we conducted molecular phylogenetic analyses based on sections of multiple loci, including the large subunit of nuclear ribosomal RNA gene (nLSU), the internal transcribed spacer regions (ITS), the small subunit of nuclear ribosomal RNA gene (SSU) and the second-largest subunit of RNA polymerase II gene (RPB2), as well as morphological studies, of collected samples of Hydnellum from China. We also inferred Maximum Likelihood and Bayesian phylogenies for the order Thelephorales from the dataset of the combined nLSU and ITS. This study has revealed the phylogenetic position of Hydnellum in the order Thelephorales, and phylogenetically confirmed ten major clades in Thelephorales; Twentynine taxa are proposed, described or reported, including 10 new subgenera (Hydnellum subgenus Hydnellum, subg. Caesispinosum, subg. Croceum, subg. Inflatum, subg. Rhizomorphum, subg. Scabrosum, subg. Spongiosum, subg. Subindufibulatum, subg. Violaceum and subg. Zonatum), 11 new species (Hydnellum atrorubrum, H. atrospinosum, H. bomiense, H. brunneorubrum, H. fibulatum, H. granulosum, H. inflatum, H. rubidofuscum, H. squamulosum, H. sulcatum and H. yunnanense), 3 newly recorded species (H. caeruleum, H. peckii and H. spongiosipes) and 5 notable specimens (Hydnellum sp 1, H. sp 2, H. sp 3, H. sp 4 and H. sp 5). A classification system based on the morphological characteristics (especially the hyphal structure types) and molecular analyses is proposed to accommodate most species in Hydnellum. The distinguishing characters of the subgenera and the new species with their closely related taxa are discussed. A key to the species of Hydnellum from China is provided.

Keywords: nLSU + ITS + SSU + RPB2; stipitate hydnoid fungi; taxonomy; new taxa; Thelephorales

1. Introduction

The genus *Hydnellum*, together with *Bankera*, *Phellodon* and *Sarcodon*, are a homogenous group of soil-inhabiting Basidiomycota (with the common characteristic of a hymenophore with a spinulose hymenium) that belongs to the Bankeraceae, Thelephorales [1,2].

All species of Bankeraceae are considered ectomycorrhizal and are associated with woody plants, mainly members of Pinaceae and Fagaceae [3–7], and colonize natural or relatively undisturbed forests [8]. These fungi can absorb organic substances from host plants and also transport nutrients and water from the soil to the plants, which improves the stability of forest ecosystems [9,10]. In addition, some species of *Hydnellum* have important medicinal functions, including cholesterol-lowering, antioxidant, anti-inflammatory, anti-tumor, immune enhancement, etc. [11]. For instance, Lee et al. [12] suggested that *H. concrescens* extracts prevents the expression of NDV-HN glycoprotein on the cell surface by



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Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). inhibiting the activity of α -glucosidase, thus exhibiting anti-viral function. This can be on par with the health benefit potentials of herbal plant infusions [13].

Due to substantial declines in abundance, they have become the focus of increasing conservation concern [14–16]. This is mainly attributed to the effect of habitat loss, aerial pollution, nitrogen deposition and soil acidification [17–21]. Stipitate hydnoid fungi, as symbols for the recent decline of ectomycorrhizal fungi, have been redlisted in, e.g., Norway, Poland, Germany, and the Netherlands [7,14,18,19,22–24]. Furthermore, an action plan for 14 rare species of hydnoid fungi has been announced to provide strategic management for their future conservation in the UK [25].

The genus *Hydnellum* is characterized by single to gregarious or coalescent pileate, stipitate basidiocarps, spinous hymenophore, corky to woody, not duplex to duplex, azonate to zonate context, uninflated to inflated generative hyphae, with or without clamp connections, and brown, irregularly ellipsoid to globose, tuberculate basidiospores. Some species display olivaceous or blue-green colours with KOH [1,2,5,26,27]. However, differentiation between closely related species within *Hydnellum* becomes significantly difficult on account of their macromorphological polymorphism caused by growing around obstacles or fusing to other adjacent basidiocarps [2,27]. Therefore, molecular sequence data are very important in identifying them. Molecular evidence has confirmed that Hydnellum has a close phylogenetic affiliation with the genus Sarcodon, and both genera aggregated in the same clade, named the "Hydnellum-Sarcodon lineage" [28–30]. Furthermore, the phylogenetic analysis of Hydnellum and Sarcodon according to Baird et al. [27] suggested that the generic limits need reassessment. To revise the generic limits and make genera monophyletic, Larsson et al. [31] moved 12 species from Sarcodon to Hydnellum, resulting in the generic circumscription of *Hydnellum* being amended. Morphologically, basidiospore size appears to separate the genera in most cases.

Most of the described species of *Hydnellum* are distributed to North America [2,27,32–34] and Europe [1,6,35,36], with a few species reported from Singapore, India, Australia, and New Guinea [26]. About 61 species have been described and transferred to the genus according to Index Fungorum (http://www.indexfungorum.org/ (accessed on 1 August 2021)) and MycoBank; however, only three taxa have been previously reported from China, and detailed molecular studies have not been performed [37]. Some specimens of this genus collected from China were identified as *H. aurantiacum*, *H. ferrugineum* and *H. suaveolens* based solely on morphological characteristics. However, molecular methods revealed that these specimens are misidentified, and the specimens need to be re-identified.

Numerous *Hydnellum* specimens have been collected from field investigations on stipitate hydnoid fungi in China during the past two decades. During the study of these specimens, twenty-nine new taxa have been identified using morphological characters and phylogenetic analyses of nuc rDNA ITS1-5.8S-ITS2 combined with nuc 28S rDNA, nuc 18S rDNA and nuc RPB2 rDNA sequences. In this paper, we present these taxa with illustrated morphological descriptions, phylogeny and comparison with related and/or similar taxa, a key and classification system.

The aims of this study are: (1) To describe the new taxa of *Hydnellum* from China and confirm or propose infrageneric subdivision (new subgenera, new species and newly recorded species) based on morphological and phylogenetic analyses; (2) To provide a classification system using hyphal structure types, molecularly supported clades and morphological characteristics within *Hydnellum* and *Sarcodon*; and (3) To confirm the phylogenetic position of *Hydnellum* within the Thelephorales.

2. Materials and Methods

2.1. Morphological Studies

Specimens were deposited at the herbarium of the Institute of Applied Ecology, Chinese Academy of Sciences (IFP). Microscopic procedures followed Mu et al. [38]. Structures were examined microscopically from sections mounted in Cotton Blue (CB): 0.1 mg aniline blue dissolved in 60 g pure lactic acid; CB+ = cyanophilous, CB- = acyanophilous. Amy-

loid and dextrinoid reactions were tested in Melzer's reagent (IKI): 1.5 g KI (potassium iodide), 0.5 g I (crystalline iodine), 22 g chloral hydrate, 20 mL distilled water; IKI— = neither amyloid nor dextrinoid reaction. Sections were mounted in 5% KOH and studied at magnifications up to $1000 \times$ using a Nikon Eclipse E600 microscope (Tokyo, Japan) with phase contrast illumination. Dimensions were measured by the ruler in the eyepiece, with accuracy within 0.1 µm. In presenting basidiospore size ranges, 5% of the measurements at each end of the range are given in parentheses. The following abbreviations are used in the text: Lm = mean spore length, Wm = mean spore width, Q = range of length/width ratios for specimens studied, and n = total number of basidiospores measured from a given number of specimens. The surface morphology for the basidiospores was observed with a Phenom Prox scanning electron microscope (ESEM, Phenom Prox, FEI, The Netherlands) at an accelerating voltage of 20 kV. A thin layer of gold was coated on the samples to avoid charging. Special color terms are from Rayner [39] and Munsell [40].

2.2. Molecular Procedures and Phylogenetic Analyses

Fungal taxa and strains used in this study are listed in Table 1. Phire Plant Direct PCR Kit (Thermo Fisher Scientific, Waltham, MA, USA) procedures were used to extract total genomic DNA from the basidiocarps. Polymerase chain reactions (PCR) was performed on a Bio-Rad T100TM Thermal cycler (Bio-RAD Inc., Hercules, CA, USA). Amplification reactions were performed in a 30 μ L reaction mixture using the following final concentrations or total amounts: 0.9 μ L template DNA, 15 μ L of 2× Phire Plant PCR buffer, 1.5 μ L of each primer, 0.6 µL Phire HS II DNA Polymerase, and 10.5 µL ddH₂O (double distilled water). Primer sequences for the used genes are provided in Table 2. The PCR lthermal cycling program condition was set as follows: initial denaturation at 98 °C for 5 min, followed by 39 cycles at 98 °C for 30 s, \times °C (the annealing temperatures for LROR/LR7, ITS1-F/ITS4, NS1/NS4, and bRPB2-6F/bRPB2-7.1R were 47.2 °C, 57.2 °C, 48 °C and 57.2 °C, respectively) for 30 s, 72 °C for 30 s, and a final extension at 72 °C for 1 min. PCR amplification was confirmed on 1% agarose electrophoresis gels stained with ethidium bromide [41]. DNA sequencing was performed at the Beijing Genomics Institute (BGI). All newly generated sequences were submitted to GenBank. Additional LSU rDNA, ITS rDNA, SSU rDNA and RPB2 rDNA sequences in the dataset used to establish phylogenetic relationships were downloaded from GenBank (http://www.ncbi.nlm.nih.gov/genbank/php (accessed on 10 August 2021)) and UNITE (https://unite.ut.ee/index.php (accessed on 10 August 2021)) (Table 1). Nuclear ribosomal RNA genes were used to determine the phylogenetic position of the new species. After PCR amplification, the products were sequenced in both directions and the sequences were assembled using DNAMAN 8.0. DNA sequences were aligned using MAFFT 7.110 [42]. To ensure the repeatability of the results, alignments were not manually adjusted. The best-fit evolutionary models selected by jmodeltest-2.1.10 for genes were GTR+I+G (nLSU), K80+G (ITS1), K80 (5.8S), JC+G (ITS2), TrN+I+G (SSU), K80+G (RPB2) in the first dataset (Hydnellum and Sarcodon dataset) and GTR+I+G (nLSU), K80+G (ITS1), K80+G (5.8S), K80+G (ITS2) in the second dataset (Thelephorales dataset). These models were applied in Bayesian analyses. All gaps were treated as missing data. Maximum Likelihood (ML) analysis was performed in RAxML v8.2.4 with GTR+I+G model [43]. The best tree was obtained by executing 100 rapid bootstrap inferences and thereafter a thorough search for the most likely tree using one distinct model/data partition with joint branch length optimization [44]. Bayesian analyses with MrBayes 3.2.4 [45] implementing the Markov Chain Monte Carlo (MCMC) technique and parameters predetermined with MrMODELTEST2.3 [46,47] were performed. Four simultaneous Markov chains were run starting from random trees, keeping one tree every 100th generation until the average standard deviation of split frequencies was below 0.01. The value of burn-in was set to discard 25% of trees when calculating the posterior probabilities. Bayesian posterior probabilities were obtained from the 50% majority rule consensus of the trees kept. Then the FigTree v1.3.1 were used to visualize the resulting trees.

Table 1. Voucher numbers, geographic origins and GenBank accession numbers for the specimens included; sequencesproduced in this study are in bold.

	Geographic		GenBank Accessions No.			
Species	Origin Voucher Number		SSU	ITS	nLSU	RPB2
Amaurodon aquicoeruleu Agerer	Australia	UK452	-	AM490944	AM490944	_
A. sumatranus Miettinen & Kõljalg	Indonesia	O. Miettinen5877	-	AM490943		-
A. viriais (Alb. & Schwein.) J. Schrot.	Russia	KHLarsson1494/b TA A149664	_	MK602707 AM490942	MK602707 AY586625	_
Bankera fuligineoalba (J.C. Schmidt)	Courdan	EL		MI(00709	MIC(00709	
Coker & Beers ex Pouzar	Sweden	ELarsson400-13	_	MK602708	NIK602708	_
B. fuligineoalba	Estonia	TAA152454	-	- MK (02700	AY586635	_
B. moluscens (Alb. & Schwein.) Pouzar B. molascens	Finland	RGC14-033	_	MH310793	MIK602709	_
Boletopsis grisea (Peck) Bondartsev &	Guadan	LIDS E 120282		MNI526751	MNI525646	
Singer	Sweden	0131-120382	—	MIN330731	NIN333040	—
B. grisea P. laucomelama (Porc.) Found	Spain	AH 42971 Krikorow140012	—	MN536747 MK602710	MN535642 MK602710	_
B. nothofagi I.A. Cooper & P. Leonard	New Zealand	PDD:96007	_	IO417193	-	_
Hydnellum amygdaliolens (Rubio Casas,	Therian			·~		
Rubio Roldán & Català) E. Larss., K.H.	Peninsula	SC-2011	-	JN376763	-	-
H. atrorubrum	China	Wei8315	_	MW579937	_	_
H. atrorubrum	China	Wei8261	MW579910	MW579936	MW579884	_
H. atrospinosum	China	Yuan6495	MW579911	MW579938	MW579885	-
H. atrospinosum	China	Yuan6514 Yuan6520	MW579913	MW579940	MW579886	-
	Ciiiia	EBendiksen177-	10100 37 9912	NIW 579959	-	-
H. aurantiacum (Batsch) P. Karst.	Norway	07	-	MK602712	MK602712	-
H. aurantiacum	Norway	OF29502	-	MK602713	MK602713	-
H. auratile (Britzelm.) Maas Geest.	Norway	OF242763 OF204005	—	MK602715 MK602714	MK602715 MK602714	—
H. bomiense	China	Yuan 13759	MW579914	MW579941	MW579887	OK254206
H. bomiense	China	Yuan 13767	MW579915	MW579942	_	_
H. bomiense	Estonia	TUF100611	_	UDB003287	-	_
H. bomiense H brunneorubrum	Costa Rica	IUF100057 Yuan12997	_ MW579917	UDB003286 MW579944		 OK254217
H. brunneorubrum	China	Yuan14339	MW579916	MW579943	MW579888	OK254217 OK254216
H. brunneorubrum	China	Yuan14668	MW579918	MW579945	MW579890	OK254218
H. caeruleum (Hornem.) P. Karst.	Norway	EBendiksen584- 11	-	MK602719	MK602719	_
H. caeruleum	Norway	EBendiksen575-	_	MK602718	MK602718	_
H. caeruleum	China	Wei1474a	_	MW579965	-	_
H. chrysinum K.A. Harrison	_	SC071	-	KJ534291	_	-
H. coactum Y.H. Mu & H.S. Yuan	China	Wei8094	-	MN846278	MN846287	-
H. complicatum Banker	USA	REB-71	_	KC571711	WIIN040200	_
H. complicatum	USA	REB-329	-	KC571712	-	-
H. concrescens (Pers.) Banker	USA	SEW 88	_	AY569025	-	_
H. concrescens H. cristatum (Bres.) Stalpers	Mexico	GO-2009-204 RFB-169	_	KC152116 IN135174	-	_
H. cristatum	USA	REB-88	_	KC571718	_	_
H. cumulatum K.A. Harrison	Finland	TU115384	_	UDB011871	UDB011871	_
H. cumulatum	Estonia	TU111191	-	UDB032402	-	-
H. cyanopoatum K.A. Harrison H. diabolus Banker	USA Canada	5EW 85 KAH13873	_	AY 569027 AF351863	_	_
H. dianthifolium Loizides	Cyprus	ML61211HY	_	KX619419	_	_
H. dianthifolium	Ítaly	ML902162HY	_	KX619420	-	_
H. earlianum Banker	USA	REB-75	-	KC571724	-	-
H. earlianum H fagiscabrosum A M Ainsw & Nitare	USA Sweden	GB-0195621	_	JIN135179 MW144293		_
H. fagiscabrosum	Sweden	GB-0195622	_	MW144296	MW144296	_
H. fennicum (P. Karst.) E. Larss, K.H.	Norway	OF242833	_	MK602738	MK602738	_
Larss. & Köljalg	Norway	OE204087		MK602727	MK 602727	
H. ferrugineum (Fr.) P. Karst	Norway	OF294087 OF297319	_	MK602720	MK602720	_
H. ferrugineum	Sweden	ELarsson197-14	_	MK602722	MK602722	_
H. ferrugipes Coker	USA	REB-176	-	KC571727	-	-
H. ferrugipes H. fibulatum	USA	KEB-68 Vuan14646	- MW570026	JN135176 MW579957	_	_
H. fibulatum	China	Yuan14656	MW579927	MW579958	_	_
H. fuligineoviolaceum (Kalchbr.) E.	Sweden	BNvlep130918	_	MK602741	MK602741	_
Larss., K.H. Larss. & Kõljalg	Cours 1	L A 120010	_	MIZ 600740	MIX 600740	—
н. juligineoviolaceum H. fuscoindicum (К.А. Harrison) F	Sweden	LA120010	-	WIN0U2/40	WIN002/40	_
Larss., K.H. Larss. & Kõljalg	USA	OSC 113641	-	EU669230	EU669280	-
H. fuscoindicum	USA	OSC 107844	-	EU669229	EU669279	_
H. glaucopus (Maas Geest. & Nannf.) E. Larss, K.H. Larss & Kõlialo	Sweden	JNitare060916	-	MK602744	MK602744	_
Larso, 1.11. Larso. & Royarg						

<u> </u>	Geographic	Voucher	GenBank Accessions No.			
Species	Origin	Number	SSU	ITS	nLSU	RPB2
H. glaucopus	Sweden	Edvinson110926	_	MK602745	MK602745	_
H. geogenium (Fr.) Banker	-	AFTOL-ID 680	AY752971	DQ218304	AY631900	DQ408133
H. geogenium	Norway	OF296213	-	MK602724	MK602724	-
H. gracilipes (P. Karst.) P. Karst.	Sweden	ELarsson219-11	-	MK602726	MK602726	-
H. gracilipes	Sweden	GB-0113779	-	MK602727	MK602727	-
H. granulosum	China	Yuan12213a	MW579921	MW579948	MW579893	OK254213
H. granulosum	China	Yuan12213b	MW579920	MW579947	MW579892	OK254212
H. grosselepidotum Y.H. Mu & H.S. Yuan	China	Wei8120	-	MN846274	MN846283	-
H. grosselepidotum	China	Wei8075	-	MN846276	MN846285	_
H. illudens (Maas Geest.) Nitare	Sweden	GB-0195819	_	MW144341	MW144341	_
H. illudens	Norway	O-F-242769	_	MW144335	MW144335	-
H. inflatum	China	Wang80	MW579922	MW579949	MW579894	OK254210
H. inflatum	China	Shi506	MW579923	MW579950	MW579895	OK254211
H. joeides (Pass.) E. Larss., K.H. Larss & Kõljalg	Sweden	KHjortstam17589	_	MK602750	MK602750	-
H. joeides	Sweden	Nitare110829	-	MK602751	MK602751	-
H. lepidum (Maas Geest.) E. Larss., K.H. Larss. & Kõljalg	Sweden	JNitare110829	-	MK602754	MK602754	_
H. lepidum	Sweden	RGCarlsson10- 065	-	MK602752	MK602752	-
H. lidongensis Y.H. Mu & H.S. Yuan	China	We8365	_	MN846280	MN846289	-
H. lidongensis	China	Wei8329	-	MN846281	MN846290	-
<i>H. lundellii</i> (Maas Geest. & Nannf.) E. Larss., K.H. Larss. & Kõljalg	Norway	OF242639	-	MK602759	MK602759	-
H. lundellii H. martioflazyum (Spell K. A	Norway	OF295814	-	MK602760	MK602760	-
Harrison & H.A.C. Jacks.) E.	Norway	OF242435	-	MK602762	MK602762	-
H. martioflavum	Norway	OF242872	_	MK602761	MK602761	_
H. mirabile (Fr.) P. Karst.	Sweden	SLund140912	_	MK602730	MK602730	_
H. mirabile	Sweden	ELarsson170-14	_	MK602729	MK602729	_
H. nemorosum A.M. Ainsw. & E. Larss	Norway	O-F-242352	_	MW144372	MW144372	_
H. nemorosum	Sweden	GB-0195631	_	MW144373	MW144373	_
<i>H. varvum</i> Banker	USA	REB-131	_	IN135187	_	_
H. parvum	USA	REB-392	_	KC571717	_	_
H. peckii Banker	Norway	SSvantesson328	_	MK602731	MK602731	_
H. peckii	Sweden	ELarsson174-14	_	MK602732	MK602732	_
H. neckii	China	Yuan13708	MW579931	MW579966	MW579905	OK254214
H. neckii	China	Yuan13720	MW579932	MW579967	MW579906	OK254215
H nineticola K A Harrison	USA	REB-49	_	KC571733	_	_
H nineticola	USA	REB-43	_	IN135175	_	_
H nineratum Coker ex Maas Geest	USA	RFB-332	_	IN135173	_	_
H nineratum	USA	REB-304	_	KC571723	_	_
H regium K A Harrison	USA	SEW 93	_	AV569031	_	_
H roseoziolaceum Nitaro	Sweden	CB-0195936	_	MW144374	MW144374	_
H roseoviolaceum	Sweden	GB-0195950 GB-0195687	_	MW144375	MW144374	_
H mhidofuscum	China	SD-0195007 Vuon14561	- MW570024	MW570051	MW570806	- OK254207
H mbidafuaaum	China	Vuan1/507	MW57002F	MM/570052	MM570207	OK25420/
H rubidofuscum	China	1ud111430/ V112011651	111113/9923	MW/570052	MM570000	OK254200
H controcum (Er.) E Lorea VU	China	1ua1114004	-	101 00 37 9933	141 44 37 9090	UK234209
Larss. & Kõljalg	Norway	OF360777	-	MK602765	MK602765	
H. scabrosum	Norway	OF292320	-	MK602766	MK602766	
H. scabrosellum Nitare	Sweden	GB-0195792	-	MW 144380	MW 144380	-
п. scabrosellum	Sweaen	GD-0195807	_	IVI VV 144381	10100144381	-
п. scieropoaium К.А. Harrison	USA	KEB-3	-	JIN135186	-	-

Table 1. Cont.

Species	Geographic Voucher		GenBank Accessions No.			
Species	Origin	Number	SSU	ITS	nLSU	RPB2
H. scleropodium	USA	REB-352	_	KC571740	_	_
H. scrobiculatum (Fr.) P. Karst.	USA	REB-78	-	JN135181	-	-
H. spongiosipes (Peck) Pouzar	USA	REB-107	-	KC571743	-	-
H. spongiosipes	USA	REB-52	-	JN135184	-	-
H. spongiosipes	China	Yuan14517	MW579933	MW579968	MW579907	OK254219
H. squamulosum	China	Yuan13615	-	MW579954	-	-
H. squamulosum	China	Yuan13625	-	MW579956	MW579899	OK254204
H. squamulosum	China	Yuan13743	-	MW579955	-	OK254203
H. suaveolens (Scop.) P. Karst.	Sweden	ELarsson8-14	-	MK602735	MK602735	-
H. suaveolens	Norway	SSvantesson877	-	MK602736	MK602736	-
H. subsuccosum K.A. Harrison	USA	SEW 55	-	AY569033	-	-
H. subsuccosum	USA	REB-10	-	JN135178	-	-
H. sulcatum	China	Yuan14521	MW579930	MW579961	MW579902	OK254202
H. sulcatum	China	Yuan14649	MW579929	MW579960	MW579901	-
H. sulcatum	China	Yuan14660	MW579928	MW579959	MW579900	OK254201
H. yunnanense	China	Yuan14386	_	MW579962	MW579903	OK254199
H. yunnanense	China	Yuan14396	_	MW579963	MW579904	OK254200
H. yunnanense	China	Shi212	-	MW579964	-	-
H. underwoodii (Banker) E. Larss.,				IN110E100		
K.H. Larss. & Kõljalg	USA	KED-358	_	JIN135189	-	-
H. underwoodii H. garcinalle (Er.) E. Large, K. H.	USA	REB-119	-	KC571782	-	_
Larss. & Kõljalg	Sweden	057	-	MK602771	MK602771	-
H. versipelle	Sweden	RGCarlsson11- 08	-	MK602772	MK602772	_
Hydnellum sp 1	China	Shi164	-	MW579969	-	-
Hydnellum sp 2	China	Yuan14387	MW579934	MW579970	MW579908	-
Hydnellum sp 3	China	Yuan14388	-	MW579971	-	-
Hydnellum sp 4	China	Wang295	-	MW579972	-	-
Hydnellum sp 5	China	Yuan14594	MW579935	MW579973	MW579909	OK254205
<i>Lenzitopsis daii</i> L.W. Zhou &	China	Yuan 2959	_	IN169799	IN169795	_
Koljalg I daji	China	Yuan2952	_	IN169798	IN169794	_
L. unit L. orucedri Malencon & Bertault	Spain	KHI arsson15304	_	MK602774	MK602774	_
L. oxycedri	- -	UK 635	_	JN169800	JN169796	_
Odontia fibrosa (Berk. & M.A. Curtis) Kõliala	China	TU115028	-	MK602775	MK602775	
O. fibrosa	China	LL_17	_	MT678878	-	_
<i>O. sparsa</i> Yuan, Y.C. Dai & H.S.	China	Yuan10718	_	MG719980	-	_
Ω sparsa	China	Yuan10780	_	MG719979	_	_
Phellodon cf. niger	Sweden	ELarsson35-14	_	MK602782	MK602782	_
P. tomentosus (L.) Banker	Norway	EBendiksen11- 810	_	MK602781	MK602781	_
P. tomentosus	_	BG Thesis	_	_	AF518637	_
Poluozellus mariae Voitk & Kõlialg	Canada	TU117348	_	MF100831	MF100831	_
P mariae	Canada	TU117235	_	MF100826		_
P multipley (Underw) Murrill	USA	TU117350	_	MF100830	MF100830	_
P multinlex	China	TU115049	_	MF100812	MF100812	_
Pseudotomentella abundiloba	Cimia N	05110010		NH 100012	NH 100012	
Svantesson	Norway	OF110312		MK290731	MK290731	
<i>P. flavovirens</i> (Höhn. & Litsch.) Svrček	Finland	KHLarsson16190	-	MK602780	MK602780	-
P. rotundispora Svantesson	Sweden	SS394	_	MK290728	MK290728	_
P. rotundispora	Sweden	SS413	-	MK290674	-	-
P. umbrinascens Svantesson	Sweden	SS335	-	MK290697	MK290697	-
Sarcodon aspratus (Berk.) S. Ito	-	-	-	DQ448877	-	-

Table 1. Cont.

Constant	Geographic	Voucher	GenBank Accessions No.			
Species	Origin	Number	SSU	ITS	nLSU	RPB2
S. aspratus	_	_	-	AF335110	-	-
S. imbricatus (L.) P. Karst.	Norway	SSvantesson355	_	MK602748	MK602748	_
S. imbricatus	Sweden	ELarsson384-10	-	MK602747	MK602747	-
<i>S. leucopus</i> (Pers.) Maas Geest. & Nannf.	Norway	OF296099	_	MK602755	MK602755	_
S. leucopus	Sweden	PHedberg080811	_	MK602757	MK602757	_
S. quercinofibulatus Pérez-De-Greg., Macau & J. Carbó	Italy	JC-20090718.2	-	JX271818	MK602773	_
S. quercinofibulatus	USA	TENN	_	MG663244	-	_
S. scabripes (Peck) Banker	Mexico	FCME:23240	-	EU293829	-	-
S. scabripes	USA	REB-351	-	JN135191	-	-
S. squamosus (Schaeff.) P. Karst.	Norway	OF295554	-	MK602769	MK602769	-
S. squamosus	Norway	OF177452	-	MK602768	MK602768	-
Steccherinum murashkinskyi (Burt) Maas Geest.	Russia	X449	-	JN710588	JN710588	_
<i>S. ochraceum</i> (Pers. ex J.F. Gmel.) Gray	Sweden	KHL11902	-	JQ031130	JQ031130	_
Thelephora ganbajun M. Zang	China	GDGM 48899	_	MF593267	MH620355	_
T. ganbajun	China	GDGM 48891	_	MF593266	MH620354	_
<i>T. iqbalii</i> Nasir & Hanif	Pakistan	MH810	_	JX241471	-	_
<i>T. terrestris</i> Ehrh.	Denmark	DMS-9327942	_	MT644883	MT644883	_
T. terrestris	Norway	ELarsson295-13	-	MK602777	MK602777	-
<i>Tomentella fuscocrustosa</i> H.S. Yuan, X. Lu & Y.C. Dai	China	Yuan11399	-	MK211712	MK446366	_
T. fuscocrustosa	China	Yuan11420	-	MK211713	MK446367	-
T. patagonica Kuhar & Rajchenb.	Argentina	BAFC52372	-	KT032090	KT032102	-
T. patagonica	Argentina	BAFC52373	-	KT032091	KT032103	-
<i>Tomentellopsis bresadoliana</i> (Sacc. & Trotter) Jülich & Stalpers	Sweden	JEH 031011	_	EU118674	EU118674	_
T. pulchella Kõljalg & Bernicchia	Norway	KHLarsson16366	_	MK602779	MK602779	-

Table 1. Cont.

Newly generated sequences in this study are in bold.

Table 2. The gene fragments, their corresponding primers and primer sequences used in this study.

Genes	Primers	Primer Sequences (5'-3')	References
	LROR	ACCCGCTGAACTTAAGC	Vilgalys & Hester 1990 [48]
nLSU	LR7	TACTACCACCAAGATCT	Vilgalys & Hester 1990 [48]
ITS	ITS1-F	CTTGGTCATTTAGAGGAAGTAA	White et al. 1990 [49]
	ITS4	TCCTCCGCTTATTGATATGC	White et al. 1990 [49]
nSSU	NS1	GTAGTCATATGCTTGTCTC	White et al. 1990 [49]
	NS4	CTTCCGTCAATTCCTTTAAG	White et al. 1990 [49]
DDDO	bRPB2-6F	TGGGGYATGGTNTGYCCYGC	Liu et al. 1999 [50]
RPB2	bRPB2-7.1R	CCCATRGCYTGYTTMCCCATDGC	Liu et al. 1999 [50]

3. Results

Phylogenetic Analyses

In the first dataset, 272 sequences derived from four gene loci (nLSU, ITS, SSU and RPB2) were used to build phylogenetic trees; 108 of them were newly generated, including 25 of nLSU, 37 sequences of ITS, 25 of SSU and 21 of RPB2. The phylogenetic construction performed with maximum likelihood and Bayesian Inference (BI) analyses for two combined datasets showed similar topology. The combined LSU-ITS-SSU-RPB2 dataset represented 70 taxa and 3629 characters after being trimmed. *Polyozellus mariae* and *P. multiplex* were used as the outgroups according to phylogenetic analysis of Thelephorales. Bayesian analysis ran for 8 million generations and resulted in an average standard deviation of split frequencies of 0.005062. The same dataset and alignment were analysed

using the ML method. The Maximum Likelihood tree is shown in Figure 1. In the phylogenetic tree, ten clades which correspond to subgenus Hydnellum, subg. Caesispinosum, subg. Croceum, subg. Inflatum, subg. Rhizomorphum, subg. Scabrosum, subg. Spongiosum, subg. Subindufibulatum, subg. Violaceum and subg. Zonatum were revealed. Twenty-eight sampled specimens formed 11 new species (Hydnellum atrorubrum, H. atrospinosum, H. bomiense, H. brunneorubrum, H. fibulatum, H. granulosum, H. inflatum, H. rubidofuscum, H. squamulosum, H. sulcatum and H. yunnanense) and clustered in a clade that comprised most species of Hydnellum. Four sampled specimens (Wei1474a, Yuan13708 and Yuan13720, Yuan14517) that were confirmed as new records from China clustered with Hydnellum caeruleum, H. peckii and *H. spongiosipes* with strong support. In addition, five notable specimens, *Hydnellum* sp 1, *Hydnellum* sp 2, *Hydnellum* sp 3, *Hydnellum* sp 4 and *Hydnellum* sp 5, formed five separate clades, and need further verification. In the second dataset, the combined ITS and nLSU gene also included sequences from 129 specimens representing 58 taxa of Thelephorales, as well as Steccherinum ochraceum and S. murashkinskyi, which were chosen as outgroups according to previous study [31]. The average standard deviation of split frequencies in the Bayesian analyses reached 0.007357 after running for 8 million generations. The calculated values based on the dataset analysed using the ML method. The Maximum Likelihood tree is shown in Figure 2. It revealed that the *Hydnellum* clade occupies an independent phylogenetic position. The Hydnellum clade is sister to the Sarcodon clade. According to the phylogenetic tree, ten major clades, Amaurodon clade, Boletopsis clade, Hydnellum clade, Lenzitopsis clade, Odontia clade, Phellodon/Bankera clade, Pseudotomentella/Polyozellus clade, Sarcodon clade, Thelephora/Tomentella clade and Tomentellopsis clade, were identified within the Thelephorales (Figure 2). Therefore, in order to use the maximum amount of genetic information when defining new species, we conducted the first dataset. Meanwhile, ITS trees of *Hydnellum* and *Sarcodon* were constructed and produced a topology similar to that generated by the first dataset (see Supplementary, Figure S1). The purpose of executing the second dataset was to demonstrate the phylogenetic position of *Hydnellum* species in the Thelephorales.



Figure 1. Cont.



Figure 1. Maximum likelihood tree illustrating the phylogeny of *Hydnellum* and *Sarcodon* based on nLSU, ITS, SSU and RPB2 sequence datasets. Branches are labeled with maximum likelihood bootstrap support greater than 50 % and Bayesian posterior probabilities greater than 0.95. Newly sequenced collections are in bold.



Figure 2. Cont.



Figure 2. Maximum likelihood phylogenetic analysis based on the nLSU and ITS sequences of Thelephorales. Branches are labeled with maximum likelihood bootstrap support greater than 50 % and Bayesian posterior probabilities greater than 0.95. Newly sequenced collections are in bold.

4. Taxonomy

Hydnellum subg. Hydnellum

MycoBank MB841191

Etymology. Hydnellum (Latin), refers to the subgenus in which the type species of the genus is located.

Included species: Hydnellum atrospinosum, H. suaveolens

Type species: Hydnellum suaveolens (Scop.) P. Karst.

Notes: This subgenus consists of the genus type *Hydnellum suaveolens* and our new species *H. atrospinosum*; they share the characteristics of dark blue context, decurrent and dark spines, clamped generative hyphae in the context and the spines trama and irregularly oblong, tuberculate basidiospores of similar size. Furthermore, both species occur in coniferous forests [27,34].

Hydnellum atrospinosum Y.H. Mu & H.S. Yuan, sp. nov. (Figure 3)



Figure 3. *Hydnellum atrospinosum.* (**a**): Basidiocarps; (**b**,**c**): SEM of basidiospores; (**d**–**f**): Microscopic structures (drawn from IFP 018516); (**d**): Section of hymenophore trama with basidia; (**e**): Hyphae from pileus context; (**f**): Basidiospores. —Scale bars: (**a**) = 1 cm; (**b**) = 3 μ m; (**c**) = 1 μ m; (**d**,**e**) = 10 μ m; (**f**) = 5 μ m.

MycoBank MB839034 *Etymology. Atrospinosum* (Latin), refers to the dark violet spines. *Type:* CHINA, Qinghai Province, Qilian County, Binggou Forest Park, ground in *Picea* forest, 8 September 2012, *H. S. Yuan*, Yuan 6520 (holotype IFP 018516).

Basidiocarps annual, solitary to gregarious or concrescent, leathery when fresh, becoming hard and light in weight upon drying; taste mild, odor fragrant when dry. Pileus irregularly ellipsoid to circular, later flabelliform or semicircular and applanate with age, up to 75 mm diam and 6–11 mm thick at center. Pileal surface light orange (5A4) to yellowish brown (5F6), concentrically zonate, scrobiculate when fresh, becoming glabrescent, rugose when dry; margin white (5A1) when fresh, brownish orange (5C6) when dry, even. Spine surface dark violet (15F4) when fresh, violet-gray (15F2) when dry; spines up to 2.5 mm long, base up to 0.4 mm diam, conical, 3-4 per mm, decurrent on stipe, without spines at pileus margin, brittle when dry. Context not duplex, up to 11 mm thick, light yellow (4A4), brownish gray (7F2) to dark violet (16F5) or dark blue (20E6), woody. *Stipe* lateral, up to 4 cm long and 3 cm diam, sometimes connate, leathery when fresh, woody upon drying, brown (6E6) to violet gray (15F2), glabrous, inside solid, cylindrical to flatted or broadened below with bulbous base when old. Hyphal structure: hyphal system monomitic; generative hyphae with clamp-connections, CB+ in slightly thick-walled hyphae, IKI-; tissues olivaceous in KOH. Context: generative hyphae hyaline, thin- to slightly thickwalled, moderately branched, clamped, straight, regularly arranged, sometimes flexuous and collapsed, mostly 3–6 µm diam. Spines: generative hyphae hyaline, thin- to slightly thick-walled, moderately branched, more or less parallel along spines, clamped, straight, 2-3 µm diam. Cystidia and cystidioles absent. Basidia clavate, thin-walled, with four sterigmata (2.1–4.2 μ m long), clamped at base, 17–45 \times 3–6 μ m; basidioles similar to basidia. Basidiospores irregular oblong or triangular, brown, thin-walled, tuberculate CB-, IKI-, $(4-)4.1-5.1(-5.5) \times (3-)3.1-3.9(-4) \mu m$, Lm = 4.6 μm , Wm = 3.2 μm , Q = 1.34-1.44 (n = 60/2); tuberculi isolated, sometimes in groups of two or more, then bi- to trifurcate in shape, up to 1 μ m long.

Additional specimens (paratypes) examined: CHINA, Qinghai Province, Qilian County, Binggou Forest Park, ground in *Picea* forest, 8 September 2012, *H. S. Yuan*, Yuan 6495 (IFP 018495, paratype); Yuan 6514 (IFP 018510, paratype).

Notes: *Hydnellum atrospinosum* and *H. suaveolens* have a close phylogenetic relationship with full support (100% in ML and 1.00 BPP). Morphologically, they both have single to gregarious basidiocarps with glabrous to rugose pileal surface, woody and dark blue context, an eccentric and terete stipe with a bulbous base, conical, decurrent and dark spines, clamped generative hyphae and irregularly oblong, tuberculate basidiospores of similar size. However, *H. suaveolens* differs from *H. atrospinosum* by longer spines (up to 6 mm vs. 2.5 mm in *H. atrospinosum*), context tissues turning light blue to green in KOH and presence of inflated hyphae in the context [27,34]. A special characteristic of *H. atrospinosum* is that the clamped generative hyphae are present in all parts of the basidiocarp; this trait can also be observed in *H. cruentum*, *H. cyanopodium*, *H. geogenium* and *H. scleropodium*. *H. cruentum* differs from *H. atrospinosum* by plushy to tomentose pileal surface, grayish blue and slightly longer spines (up to 3.5 mm vs. 2.5 mm in *H. atrospinosum*) and subglobose basidiospores [26,27,51]. *H. cyanopodium* and *H. scleropodium* obviously differs in blue spines [27,33,51]. *H. geogenium* differs in reflexed-multiplex and yellow basidiocarps, pale yellow to brown spines and subglobose basidiospores [1,27,34].

Hydnellum subg. Caesispinosum Y.H. Mu & H.S. Yuan, subgen. nov.

MycoBank MB841195

Etymology. Caesispinosum (Latin), refers to the blue spines.

Included species: Hydnellum cyanopodium, H. scleropodium

Type species: Hydnellum cyanopodium K.A. Harrison

Notes: The subgenus is composed of two American species, *Hydnellum cyanopodium* and *H. scleropodium*. Blue spines and context and fully clamped hyphae in all parts of basidiocarps are their distinctly common features. In addition, the two species both have rugose and pitted pileal surface and clamped basidia of similar size [33,34,51].

Hydnellum subg. Croceum Y.H. Mu & H.S. Yuan, subgen. nov.

MycoBank MB841196

Etymology. Croceum (Latin), refers to the orange basidiocarps.

Included species: *Hydnellum aurantiacum*, H. auratile, **H. brunneorubrum**, H. chrysinum, H. earlianum

Type species: Hydnellum aurantiacum (Batsch) P. Karst.

Notes: This subgenus includes five species, *Hydnellum aurantiacum*, *H. auratile*, *H. brunneorubrum*, *H. chrysinum* and *H. earlianum*. They often have orange basidiocarps, tomentose to matted pileal surface, yellow to orange spines and context, the monomitic hyphal system with uninflated and unclamped hyphae, clavate basidia with simple-septate at base and irregularly subglobose basidiospores [2,26,27,33,34,51].

Hydnellum brunneorubrum Y.H. Mu & H.S. Yuan, **sp. nov.** (Figure 4) MycoBank MB839036

Type: CHINA, Liaoning Province, Xinbin County, Gangshan Nature Reserve, on the ground in Fagaceous forest, 30 August 2018, *H. S. Yuan*, Yuan 12997 (holotype IFP 019384).

Basidiocarps annual, solitary to gregarious or multiple pilei overlapping and fused to form a compound cluster, soft and leathery when fresh, becoming woody and light in weight upon drying; taste mild, odor none when dry. Pileus applanate and ellipsoid to irregularly circular when young, later depressed or infundibuliform to flabelliform with age, up to 40 mm diam and 5–10 mm thick at center. *Pileal surface* brownish orange (6C8) to brownish red (10D8), azonate, pubscent to floccose when fresh, becoming matted or fibrillose to glabrous when dry; margin white (6A1) to light orange (6A5) when fresh, light orange (5A4) when dry, involute and wavy, sometimes lobed with age. Spine surface golden yellow (5B6) to light brown (7D8) when fresh, light brown (6D7) to dark brown (7F8) when dry; spines up to 4 mm long, base up to 0.3 mm diam, conical, 3–5 per mm, more or less decurrent on stipe, without spines at pileus margin, brittle when dry. Context not duplex, up to 5 mm thick, grayish orange (5B5), woody. Stipe central to lateral, up to 3 cm long and 1 cm diam, sometimes connate, leathery when fresh, woody upon drying, brownish orange (6C8) to light brown (6D7), tomentose, solid inner, cylindrical to flat or attenuate downwards with bulbous base when old. Hyphal structure: hyphal system monomitic; generative hyphae with simple-septa, occasionally encrusted, CB+ in slightly thick-walled hyphae, IKI-; tissues olivaceous in KOH. Context: generative hyphae hyaline, thin- to slightly thick-walled, frequently branched, simple-septate, straight, regularly arranged, sometimes flexuous and collapsed, mostly 4-6 µm diam. Spines: generative hyphae hyaline, thin-walled, frequently branched, more or less parallel along spines, frequently simple-septate, straight, 2-5 µm diam. Cystidia and cystidioles absent. Basidia clavate, thin-walled, with four sterigmata (2.5–5 μ m long), simple-septate at base, 12–50 \times 3–7 μ m; basidioles similar to basidia. Basidiospores irregularly ellipsoid to subglobose, brown, thinwalled, tuberculate, CB-, IKI-, $(4-)4.1-5.1(-5.2) \times (3.1-)3.2-4.6(-4.8) \mu m$, Lm = 4.9 μm , Wm = $3.9 \mu m$, Q = 1.23-1.26 (n = 60/2); tuberculi usually isolated, sometimes in groups of two or more, then bi- to trifurcate in shape, up to 0.8 µm long.

Additional specimens (paratypes) examined: CHINA, Liaoning Province, Xinbin County, Gangshan Nature Reserve, on the ground in mixed forest, 30 August 2018, *H. S. Yuan*, Yuan 12999 (IFP 019385, paratype); Yuan 13004 (IFP 019386, paratype); 2 September 2019, *H. S. Yuan*, Yuan 14339 (IFP 019387, paratype); Yuan 14340 (IFP 019388, paratype); Yuan 14341 (IFP 019389, paratype); 12 August 2020, *H. S. Yuan*, Yuan 14562 (IFP 019390, paratype); 26 August 2020, *H. S. Yuan*, Yuan 14585 (IFP 019391, paratype); 12 September 2020, *H. S. Yuan*, Yuan 14796 (IFP 019392, paratype); Yuan 14798 (IFP 019393, paratype); Yuan 14799 (IFP 019394, paratype); Benxi County, Guanmenshan National Forest Park, on the ground in mixed forest, 29 August 2020, *H. S. Yuan*, Yuan 14642 (IFP 019395, paratype); Xinbin County, Qingsongling Forest Park, on the ground in mixed forest, 5 September 2020, *H. S. Yuan*, Yuan 14668 (IFP 019396, paratype); Yuan 14688 (IFP 019397, paratype).



Figure 4. *Hydnellum brunneorubrum.* (**a**,**b**): Basidiocarps; (**c**,**d**): SEM of basidiospores; (**e**–**g**): Microscopic structures (drawn from IFP 019384); (**e**): Section of hymenophore trama with basidia; (**f**): Hyphae from pileus context; (**g**): Basidiospores. —Scale bars: (**a**,**b**) = 1 cm; (**c**) = 3 μ m; (**d**) = 1 μ m; (**e**,**f**) = 10 μ m; (**g**) = 5 μ m.

Notes: The orange basidiocarps make *Hydnellum brunneorubrum* similar to *H. aurantiacum*, *H. auratile*, *H. chrysinum* and *H. earlianum*. *H. aurantiacum* differs from *H. brunneorubrum* in bigger pileus (up to 100 mm vs. 40 mm in *H. brunneorubrum*) with colliculose and wrinkled pileal surface, longer spines (up to 5 mm vs. up to 4 mm in *H. brunneorubrum*) and longer basidiospores (6–6.7 μ m vs. 4.1–5.1 μ m in *H. brunneorubrum*) [1]. *H. auratile* differs in squamulose, concentrically zoned and occasionally black stained pileal surface, duplex stipe context with black lines in the centre and longer basidiospores (4.9–5.8 μ m vs. 4.1–5.1 μ m in *H. brunneorubrum*); in addition, *H. auratile* usually grows in coniferous forests [1,26]. *H. chrysinum* is differentiated by having duplex, slightly zonate context, context tissue turning dark olive or blackish in KOH and slightly longer basidia sterigmata (up to 6 μ m vs. up to 5 μ m in *H. brunneorubrum*) [2,33,51]. *H. earlianum* is differentiated by a larger pileus (up to 90 mm vs. 40 mm in *H. brunneorubrum*), duplex context, dark brown or black context tissues in KOH and longer basidiospores (5–6 μ m vs. 4.1–5.1 μ m in *H. brunneorubrum*) [27,32,34].

Hydnellum subg. *Inflatum* Y.H. Mu & H.S. Yuan, subgen. nov. MycoBank MB841197

Etymology. Inflatum (Latin), refers to the presence of inflated generative hyphae. Included species: *Hydnellum cristatum*, *H. granulosum*, *H. inflatum*, *H. mirabile*, *H. piperatum* Type species: *Hydnellum mirabile* (Fr.) P. Karst.

Notes: There are five species in the subgenus *Inflatum*, *Hydnellum cristatum*, *H. granulosum*, *H. inflatum*, *H. mirabile* and *H. piperatum*. The presence of inflated generative hyphae in the context of the pileus is an important feature they share. As well as, they often have yellow to brown and depressed pileus, cylindrical stipe and unclamped generative hyphae in the context and the spine trama [1,27,34].

Hydnellum granulosum Y.H. Mu & H.S. Yuan, **sp. nov.** (Figure 5) MycoBank MB839038

Etymology. Granulosum (Latin), refers to the granulose pileal surface when dry.

Type: CHINA, Sichuan Province, Guangyuan County, Tianzhaoshan National Forest Park, on the ground in *Acer* and *Cryptomeria* mixed forest, 13 August 2017, *H. S. Yuan*, Yuan 12213b (holotype IFP 019400).

Basidiocarps annual, solitary to gregarious or two to three pilei fused to form a complex pileus, soft and leathery when fresh, becoming hard and light in weight upon drying; taste acrid, odor fragrant when dry. Pileus aplanate, irregularly ellipsoid when young, later irregularly flabelliform with age, up to 50 mm diam and 5–10 mm thick at center. Pileal surface light yellow (4A4), light brown to gravish brown (9F3), azonate, granulose when dry; margin yellowish white (4A2), involute and wavy, sometimes lobed. Spine surface grayish orange (5B4) to dark brown (8F7) when dry; spines up to 2 mm long, base up to 0.3 mm diam, conical, 3–6 per mm, more or less decurrent on stipe, without spines at pileus margin, brittle when dry. Context not duplex, up to 9 mm thick, grayish orange (5B4), woody. Stipe central to lateral, up to 4 cm long and 3 cm diam, sometimes connate, hard upon drying, golden brown (5D6) to brown (6E6), rugose, solid inner, terete or attenuate or broadening downwards with bulbous base when old. *Hyphal structure:* hyphal system monomitic; generative hyphae with simple-septa, CB+ in slightly thick-walled hyphae, IKI-; tissues olivaceous in KOH. Context: generative hyphae hyaline, mostly slightly thickwalled, rarely thin-walled, occasionally branched, simple-septate, occasionally inflated, interwoven, mostly 4–7 μm diam. Spines: generative hyphae hyaline, thin- to slightly thickwalled, moderately branched, more or less parallel along spines, frequently simple-septate, straight, 3-4 µm diam. Cystidia and cystidioles absent. Basidia clavate, thin-walled, with four sterigmata (3–5 μ m long), simple-septate at base, 15–30 \times 5–9 μ m; basidioles similar to basidia. Basidiospores irregularly ellipsoid to globose, brown, thin-walled, tuberculate, CB-, IKI-, $(4-)4.1-5.1(-5.3) \times (3.2-)3.4-4.7(-4.9) \mu m$, Lm = 4.6 μm , Wm = 4.1 μm , Q = 1.12-1.13 (n = 60/2); tuberculi usually isolated, sometimes in groups of two or more, then bi- to trifurcate in shape, up to 1.2 µm long.

Additional specimens (paratypes) examined: CHINA, Sichuan Province, Guangyuan County, Tianzhaoshan National Forest Park, on the ground in *Acer* and *Cryptomeria* mixed forest, 12 August 2017, *H. S. Yuan*, Yuan 12213a (IFP 019401, paratype); 13 August 2017, *H. S. Yuan*, Yuan 12213c (IFP 019402, paratype).



Figure 5. *Hydnellum granulosum.* (**a**,**b**): Basidiocarps; (**c**,**d**): SEM of basidiospores; (**e**–**g**): Microscopic structures (drawn from IFP 019400); (**e**): Section of hymenophore trama with basidia; (**f**): Hyphae from pileus context; (**g**): Basidiospores. —Scale bars: (**a**,**b**) = 1 cm; (**c**) = 3 μ m; (**d**) = 1 μ m; (**e**,**f**) = 10 μ m; (**g**) = 5 μ m.

Notes: *Hydnellum granulosum* has a close phylogenetic relationship with *H. piperatum*. In morphology, *H. piperatum* resembles *H. granulosum* in having single to gregarious or concrescent basidiocarps, with lobed pileal margin from fused pilei or indeterminate growth, a single to fused, central to eccentric and terete to attenuate downwards stipe,

context tissue turning olivaceous in KOH, absence of clamp-connections and the presence of inflated hyphae. However, *H. piperatum* is distinguishable from *H. granulosum* by having a comparatively broader pileus (up to 150 mm vs. 50 mm in *H. granulosum*) with a scaly or squamulose and zonate pileal surface and strongly decurrent and longer spines (up to 5 mm vs. 2 mm in *H. granulosum*) [27]. The presence of inflated generative hyphae is a shared feature in *H. granulosum* and *H. mirabile*. They also have simple or concrescent basidiocarps with light yellow to brown pilei, and hard and cylindrical stipes. However, *H. mirabile* differs from *H. granulosum*), yellowish to purplish brown and longer spines (up to 5 mm vs. 2 mm in *H. granulosum*), duplex and pallid to pale brownish context and longer basidiospores (5.6–5.8 µm vs. 4.1–5.1 µm in *H. granulosum*) [1].

Hydnellum inflatum Y.H. Mu, X.H. Wang & H.S. Yuan, **sp. nov.** (Figure 6) MycoBank MB839040

Etymology. Inflatum (Latin), refers to the mostly inflated generative hyphae in the context of the pileus.

Type: CHINA, Yunnan Province, Maguan County, Dalishu Township, on the ground in Fagaceous forest, 14 October 2017, S. F. Shi, Shi 506 (holotype IFP 019403).

Basidiocarps annual, solitary to gregarious, soft and leathery when fresh, becoming corky and light in weight upon drying; taste acrid, odor fragrant when dry. Pileus depressed to aplanate and irregularly circular when young, later flabelliform with age, up to 75 mm diam and 3–8 mm thick at center. Pileal surface grayish orange (5B5) to brown (7E7), azonate, fibrous to colliculose when fresh, becoming scrobiculate when dry; margin light yellow (4A4) when fresh, orange-white (5A2) when dry, involute and wavy, often lobed with age. Spine surface white (5A1) to golden brown (5D7) when fresh, yellowish brown (5D5) to dark brown (7F8) when dry; spines up to 4 mm long, base up to 0.25 mm diam, conical, 4-5 per mm, strongly decurrent on stipe, without spines at pileus margin, brittle when dry. Context duplex, upper layer grayish yellow (4B5), loose and soft, up to 2 mm thick; lower layer pale yellow (4A3), woody, up to 3 mm thick; a dark line present between upper and lower cortical layers. Stipe central to lateral, up to 5 cm long and 2 cm diam, occasionally connate, fleshy when fresh, woody upon drying, light brown (6D7), smooth to rugose, inside solid, context with a dark line present at centre, cylindrical or attenuate downwards with bulbous base when old. Hyphal structure: hyphal system monomitic; generative hyphae with simple-septa, CB+ in slightly thick-walled hyphae, IKI-; tissues slightly olivaceous in KOH. Context: generative hyphae hyaline, thin- to slightly thick-walled, occasionally branched, simple-septate, mostly inflated, interwoven, mostly 4-8 µm diam. Spines: generative hyphae hyaline, thin- to slightly thick-walled, moderately branched, more or less parallel along spines, frequently simple-septate, straight, 2-5 µm diam. Cystidia and cystidioles absent. Basidia clavate, thin-walled, with four sterigmata (3–5 μ m long), simple-septate at base, 19–33 \times 4–6 μ m; basidioles similar to basidia. Basidiospores irregularly ellipsoid to globose, brown, thin-walled, tuberculate, CB-, IKI-, $(4-)4.2-5(-5.1) \times (3.2-)3.8-4.3(-5) \ \mu\text{m}, \ \text{Lm} = 4.8 \ \mu\text{m}, \ \text{Wm} = 4 \ \mu\text{m}, \ Q = 1.18-1.2 \ (n = 60/2);$ tuberculi usually isolated, sometimes in groups of two or more, then bi- to trifurcate in shape, up to 1.2 µm long.

Additional specimens (paratypes) examined: CHINA, Yunnan Province, Maguan County, Mabai Town, Caiyuanzi Village, on the ground, 5 August 2017, *J. Wang*, Wang 80 (IFP 019404, paratype); Wang 82 (IFP 019405, paratype); on the way from Dalishu Township to Damagu Village, on the ground in Fagaceous forest, 6 August 2017, *S. F. Shi*, Shi 150 (IFP 019406, paratype); Shi 160 (IFP 019407, paratype).



Figure 6. *Hydnellum inflatum.* (**a**,**b**): Basidiocarps; (**c**,**d**): SEM of basidiospores; (**e**–**g**): Microscopic structures (drawn from IFP 019403); (**e**): Section of hymenophore trama with basidia; (**f**): Hyphae from pileus context; (**g**): Basidiospores. —Scale bars: (**a**,**b**) = 1 cm; (**c**) = 3 μ m; (**d**) = 1 μ m; (**e**,**f**) = 10 μ m; (**g**) = 5 μ m.

Notes: *Hydnellum inflatum* is characterized by the presence of inflated generative hyphae, which makes it similar to *H. cristatum*, *H. granulosum*, *H. mirabile* and *H. piperatum*. This is an important feature that distinguishes from other species. However, *H. cristatum* can be differentiated from *H. inflatum* by the larger pileus (up to 100 mm vs. 75 mm in *H. inflatum*) with tomentose to matted pileal surface, longer spines (up to 5 mm vs. 4 mm in *H. inflatum*), brown context tissues in KOH, bigger basidia (34–46 × 8–9 μ m vs.

19–33 × 4–6 µm in *H. inflatum*) and basidiospores (5–6 × 4–5 µm vs. 4.2–5 × 3.8–4.3 µm in *H. inflatum*) [27,34]. *H. granulosum* differs in granulose pileal surface, not duplex context, mostly slightly thick-walled hyphae in the context and shorter spines (up to 2 mm vs. up to 4 mm in *H. inflatum*). *H. mirabile* differs in ochraceous yellow and olive brown pileus, yellowish to purplish brown spines and longer spores (5.6–5.8 µm vs. 4.2–5 µm in *H. inflatum*) [1]. *H. piperatum* differs in umbilicate and greatly pileus (up to 100 mm vs. 75 mm in *H. inflatum*), red haired to sunburn slightly longer spines (up to 5 mm vs. up to 4 mm in *H. inflatum*) and inflated to cylindrical hyphae in the spines [27].

Hydnellum subg. *Rhizomorphum* Y.H. Mu & H.S. Yuan, subgen. nov. MycoBank MB841198

Etymology. Rhizomorphum (Latin), refers to the rhizomorphs-like stipe.

Included species: *Hydnellum gracilipes*, *Hydnellum* **sp 1**

Type species: Hydnellum gracilipes (P. Karst.) P. Karst.

Notes: *Hydnellum gracilipes* and our notable specimen *Hydnellum* sp 1 comprise the subgenus *Rhizomorphum*. Rhizomorph-like stipe are their typical common characteristics. Besides, both species have the monomitic hyphal system with simple-septate generative hyphae [35].

Hydnellum sp 1

Basidiocarps annual, solitary to gregarious or coalescent. *Pileus* flabelliform to irregularly circular. *Pileal surface* deep red (10C8) to violet-brown (10F8) and felted. *Spines* white (10A1) to brownish red (10D7) and more or less decurrent, up to 1.5 mm long. *Rhizomorphs* stipe-like. *Hyphal system* monomitic, generative hyphae simple-septa. *Basidia* clavate, with four sterigmata. *Basidiospores* irregularly subglobose to globose, tuberculate, (4–)4.1–4.9(–5) \times (3.1–)3.2–4(–4.1) µm.

Specimen examined: CHINA, Yunnan Province, Maguan County, on the way from Dalishu Township to Damagu Village, on the ground of angiosperm forest, 6 August 2017, *S. F. Shi*, Shi 164 (IFP 019436).

Hydnellum subg. *Scabrosum* Y.H. Mu & H.S. Yuan, subgen. nov. MycoBank MB841199

Etymology. Scabrosum (Latin), refers to the pileal surface with scabrosity.

Included species: Hydnellum amygdaliolens, H. coactum, H. fagiscabrosum, H. fennicum, H. grosselepidotum, H. illudens, H. lepidum, H. lidongensis, H. nemorosum, H. scabrosellum, H. scabrosum, H. underwoodii

Type species: Hydnellum scabrosum (Fr.) E. Larss., K.H. Larss. & Kõljalg

Notes: There are twelve species, namely *Hydnellum amygdaliolens*, *H. coactum*, *H. fagiscabrosum*, *H. fennicum*, *H. grosselepidotum*, *H. illudens*, *H. lepidum*, *H. lidongensis*, *H. nemorosum*, *H. scabrosellum*, *H. scabrosum* and *H. underwoodii* in this subgenus characterised by planar to depressed and brown pileus, azonate pileal surface with scabrosity, variously-brown spines, not duplex and yellow to orange context, inflated and unclamped generative hyphae and irregularly ellipsoid to globose basidiospore [1,2,26,27,32–34,38,51–54].

Hydnellum subg. Spongiosum Y.H. Mu & H.S. Yuan, subgen. nov.

MycoBank MB841200

Etymology. Spongiosum (Latin), refers to the spongy pileal surface.

Included species: *Hydnellum ferrugineum*, *H. pineticola*, *H. spongiosipes*, *Hydnellum*

sp 2

Type species: *Hydnellum ferrugineum* (Fr.) P. Karst.

Notes: This subgenus includes *Hydnellum ferrugineum*, *H. pineticola*, *H. spongiosipes* and *Hydnellum* sp 2; they all have planoconvex to depressed and brown pileus with spongy pileal surface, purplish brown spines, the monomitic hyphal system with simple-septate generative hyphae and irregularly subglobose basidiospores [1,2,27,34].

Hydnellum spongiosipes (Peck) Pouzar, Česká Mykol. 14(2): 130 (1960)

Hydnum spongiosipes Peck, Ann. Rep. Reg. N.Y. St. Mus. 50: 111 (1898) (1897)

Basidiocarps single to gregarious. *Pileus* flabelliform, applanate to subdepressed. *Pileal surface* pale orange (6A3) to dark brown (6F7), velutinous to very spongy, tomentose to

fibrillose. *Spines* pale orange (6A3) to dark brown (6F7), subdecurrent to decurrent, up to 6 mm long. *Stipe* central to subeccentric, terete, thick and strong. *Hyphal system* monomitic; generative hyphae simple-septate. *Basidia* clavate, with four sterigmata. *Basidiospores* irregularly subglobose, tuberculate, $(5-)5.1-6.1(-6.2) \times (4.3-)4.5-5.3(-5.8) \mu m$.

Specimen examined: CHINA, Liaoning Province, Kuandian County, Baishilazi National Nature Reserve, on the ground of *Quercus* forest, 8 August 2020, *H. S. Yuan*, Yuan 14517 (IFP 019435).

Notes: The studied sample clustered with *Hydnellum spongiosipes* (REB-107 and REB-52) in the multi-gene phylogenetic tree with strong support (90% in ML and 1.00 BPP) (Figure 1). The samples REB-107 and REB-52 both show 0.99 similarity to Yuan 14517 in ITS region. Besides this, morphological analyses also confirmed the new record, which is described in detail by Maas Geesteranus (1975) and Baird (2013). This species was recorded to occur widely in the United States and European countries and usually was found under hardwood tree [1,2,27,34].

Hydnellum sp 2

Basidiocarps annual, coalescent. *Pileus* compound, multiple pilei fused. *Pileal surface* violet-brown (10E6) and spongy-tomentose. *Spines* white (10A1) to violet-brown (10E6) and strongly decurrent, up to 2.5 mm long. *Stipe* short and connate. *Hyphal system* monomitic, generative hyphae simple-septa. *Basidia* clavate, with four sterigmata. *Basidiospores* irregularly subglobose, tuberculate, $(3.1-)4-4.3(-4.5) \times (3-)3.1-4(-4.1) \mu m$.

Specimen examined: CHINA, Yunnan Province, Nanjian County, Lingbaoshan National Forest Park, on the ground of angiosperm forest, 19 September 2019, *H. S. Yuan*, Yuan 14387 (IFP 019437).

Hydnellum subg. Subindufibulatum Y.H. Mu & H.S. Yuan, subgen. nov.

MycoBank MB841201 *Etymology. Subindufibulatum* (Latin), refers to the occasionally clamped hyphae in the context of the pileus.

Included species: Hydnellum caeruleum, H. ferrugipes, H. fibulatum

Type species: Hydnellum caeruleum (Hornem.) P. Karst.

Notes: *Hydnellum caeruleum*, *H. ferrugipes* and *H. fibulatum* make up the subgenus *Subindufibulatum* and occasional presence of clamped hyphae in the context of the pileus is the dominating trait that distinguishes them from other species. Furthermore, they have dark brown and fibrillose to colliculose pileal surface, orange stipe, context tissues turning olivaceous in KOH and the absence of clamp-connections in the spine trama [2,26,27].

Hydnellum caeruleum (Hornem.) P. Karst., [as '*coeruleum*'], Meddn Soc. Fauna Flora Fenn. 5: 41 (1879)

Hydnum caeruleum Hornem., Fl. Danic. 8(23): 7, tab. 1374 (1808)

Basidiocarps single to gregarious or concrescent. *Pileus* convex to plane. *Pileal surface* pastel yellow (4A4) to dark blonde (5D4), tomentose and colliculose. *Spines* decurrent, up to 6 mm long, orange-white (5A2) to dark brown (6F8). *Stipe* central and terete. *Hyphal system* monomitic; most of the generative hyphae with simple-septa, rarely with clamps. *Basidia* clavate, with four sterigmata. *Basidiospores* irregularly subglobose, tuberculate, $(4.9-)5-6(-6.1) \times (4-)4.1-4.9(-5) \ \mu m$.

Specimen examined: CHINA, Xinjiang Autonomous Region, Huocheng County, Guozigou Forest Park, on the ground in *Picea* forest, 18 August 2004, *Y. L. Wei*, Wei 1474a (IFP 019432).

Notes: The phylogenetic analyses showed that the studied sample matched with *Hydnellum caeruleum* (EBendiksen584-11) with full support (100% in ML and 1.00 BPP) (Figure 1). ITS sequence BLAST also revealed it is 100% identical to *H. caeruleum*. Besides, our collection shares identical characters with *H. caeruleum* described by Maas Geesteranus [26] in morphology. This is the first report of this species from China.

Hydnellum fibulatum Y.H. Mu & H.S. Yuan, sp. nov. (Figure 7)



Figure 7. *Hydnellum fibulatum.* (**a**,**b**): Basidiocarps; (**c**,**d**): SEM of basidiospores; (**e**–**g**): Microscopic structures (drawn from IFP 019398); (**e**): Section of hymenophore trama with basidia; (**f**): Hyphae from pileus context; (**g**): Basidiospores. —Scale bars: (**a**,**b**) = 2 cm; (**c**) = 3 μ m; (**d**) = 1 μ m; (**e**,**f**) = 10 μ m; (**g**) = 5 μ m.

MycoBank MB839037

Etymology. Fibulatum (Latin), refers to the generative hyphae with occasional clamp-connections.

Type: CHINA, Liaoning Province, Benxi County, Guanmenshan National Forest Park, on the ground in *Quercus* forest, 29 August 2020, *H. S. Yuan*, Yuan 14656 (holotype IFP 019398).

Basidiocarps annual, solitary to gregarious, soft and leathery when fresh, becoming woody and light in weight upon drying; taste mild, odor none when dry. Pileus applanate, circular when young, later flabelliform with age, up to 45 mm diam and 3-7 mm thick at center. Pileal surface light brown (7D7) to dark brown (8F4), obscurely zonate, pubescent when fresh, becoming fibrillose, rugose when dry; margin white (6A1) when fresh, brown (6E6) when dry, incurved, sometimes lobed. Spine surface pinkish white (7A2) to brown (7E7) when fresh, orange-white (5A2) to brown (6E6) when dry; spines up to 1.5 mm long, base up to 0.2 mm diam, conical, 3–4 per mm, more or less decurrent on stipe, without spines at pileus margin, brittle when dry. Context not duplex, up to 5 mm thick, light brown (6D4) to dark brown (6F4), woody. Stipe central to lateral, up to 3.5 cm long and 1 cm diam, single, leathery when fresh, woody upon drying, light orange (5A5) to brown (6E7), pubescent, solid inner, cylindrical or attenuate downwards with bulbous base when old. *Hyphal structure:* hyphal system monomitic; generative hyphae with mostly simple-septa, occasionally clamped, CB+ in slightly thick-walled hyphae, IKI-; tissues olivaceous in KOH. Context: generative hyphae hyaline, slightly thick-walled, occasionally branched, simple-septate, occasionally clamped, straight, regularly arranged, sometimes flexuous and collapsed, mostly 3–5 µm diam. Spines: generative hyphae hyaline, thin- to slightly thickwalled, moderately branched, more or less parallel along spines, frequently simple-septate, straight, 2-4 µm diam. Cystidia and cystidioles absent. Basidia clavate, thin-walled, with four sterigmata (2–5 μ m long), simple-septate at base, 15–47 \times 5–9 μ m; basidioles similar to basidia. Basidiospores irregularly ellipsoid to globose, brown, thin-walled, tuberculate, CB-, IKI-, $(4.2-)4.4-5.8(-6) \times (4-)4.1-4.9(-5.1) \mu m$, Lm = 5.2 μm , Wm = 4.3 μm , Q = 1.12-1.21 (n = 60/2); tuberculi usually isolated, sometimes in groups of two or more, then bi- to trifurcate in shape, up to 1 μ m long.

Additional specimen (paratype) examined: CHINA, Liaoning Province, Benxi County, Guanmenshan National Forest Park, on the ground in *Quercus* forest, 29 August 2020, *H. S. Yuan*, Yuan 14646 (IFP 019399, paratype).

Notes: *Hydnellum caeruleum* and *H. ferrugipes* have an adjacent phylogenetic relationship with *H. fibulatum* according to the phylogenetic tree (Figure 1). *H. caeruleum* and *H. fibulatum* have similar morphological characteristics, such as a flat and velutinous pileus when immature, white pileal margin when fresh, central, terete and tomentose stipe, olivaceous context tissue in KOH, presence of occasional clamp-connections in the context and simple-septate hyphae in the spines. However, *H. caeruleum* can be distinguished by having a larger pileus (up to 80 mm vs. 45 mm in *H. fibulatum*), rough or colliculose pileal surface when mature, duplex and zonate context [2,26]. *H. ferrugipes* resembles *H. fibulatum* in having a white pileal margin when fresh, tomentose and orange to brown stipe, orange-white to brown spines when dry, regularly arranged and occasionally clamped hyphae in the context, unclamped hyphae in the spines and basidia sterigmata with similar size. However, *H. ferrugipes* differs from *H. fibulatum* in the infundibuliform pileus with pitted to subnodulose or subcolliculose pileal surface, blue-gray or grayish orange context, considerably longer spines (up to 6 mm vs. 1.5 mm in *H. fibulatum*) and wider basidiospores (5–6 µm vs. 4.1–4.9 µm in *H. fibulatum*) [27,34].

Hydnellum **subg.** *Violaceum* Y.H. Mu & H.S. Yuan, **subgen. nov.** MycoBank MB841202

Etymology. Violaceum (Latin), refers to the violaceous basidiocarps.

Included species: *Hydnellum fuligineoviolaceum*, *H. fuscoindicum*, *H. glaucopus*, *H. joeides*, *H. roseoviolaceum*

Type species: Hydnellum fuligineoviolaceum (Kalchbr.) E. Larss., K.H. Larss. & Kõljalg

Notes: Five species, *Hydnellum fuligineoviolaceum*, *H. fuscoindicum*, *H. glaucopus*, *H. joeides* and *H. roseoviolaceum*, comprise the subgenus *Violaceum*. They share the following features: violaceous basidiocarps, pileal surface with appressed scales, purplish con-

text, the presence of inflated generative hyphae and the simple-septate haphae in all tissue [1,26,27,33,53].

Hydnellum subg. Zonatum Y.H. Mu & H.S. Yuan, subgen. nov.

MycoBank MB841203

Etymology. Zonatum (Latin), refers to the concentrically zonate pileal surface.

Included species: *Hydnellum atrorubrum, H. bomiense,* H. concrescens, H. dianthifolium, H. parvum, **H. rubidofuscum**, H. scrobiculatum, **H. squamulosum**, H. subsuccosum, **H.** *sulcatum*, **H. yunnanense**, **Hydnellum sp 3**, *Hydnellum sp 4*, *Hydnellum sp 5*

Type species: *Hydnellum scrobiculatum* (Fr.) P. Karst.

Notes: The subgenus *Zonatum* contains fourteen taxa, *Hydnellum atrorubrum*, *H. bomiense*, *H. concrescens*, *H. dianthifolium*, *H. parvum*, *H. rubidofuscum*, *H. scrobiculatum*, *H. squamulosum*, *H. subsuccosum*, *H. sulcatum*, *H. yunnanense*, *Hydnellum* sp 3, *Hydnellum* sp 4 and *Hydnellum* sp 5. The concentrically zonate pileal surface is their most prominently mutual peculiarity. Additionally, the absence of clamp-connections in the context of the pileus and the spine trama is another important common feature [1,2,26,27,34,36].

Hydnellum atrorubrum Y.H. Mu & H.S. Yuan, sp. nov. (Figure 8)

MycoBank MB839032

Etymology. Atrorubrum (Latin), refers to the dark ruby red pileal surface.

Type: CHINA, Yunnan Province, Yulong County, on the ground in Fagaceous forest, 23 July 2018, *Y. L. Wei*, Wei 8261 (holotype IFP 019377).

Basidiocarps annual, solitary to gregarious or several pilei fused to form a compound pileus, soft and leathery when fresh, becoming woody and light in weight upon drying; taste slightly bitter, odor slightly fragrant when dry. Pileus applanate and flabelliform to irregularly circular when young, later depressed or subinfundibuliform to rounded with age, up to 48 mm diam and 3–8 mm thick at center. *Pileal surface* light brown (7D7) to dark ruby (12F8), usually concentrically zoned, flocculose when fresh, becoming fibrillose to glabrescent when dry; margin white (7A1) when fresh, brown (7E5) when dry, even to slightly irregular, occasionally wavy or lobed. Spine surface white (6A1) to dark brown (6F6) when fresh, grayish orange (5B4) to brown (6E8) when dry; spines up to 3.5 mm long, base up to 0.4 mm diam, conical, 3-5 per mm, decurrent to strongly decurrent on stipe, without spines at pileus margin, brittle when dry. *Context* not duplex, up to 6 mm thick, brown (6E6), corky. Stipe central, up to 3.5 cm long and 1.5 cm diam, sometimes connate, leathery when fresh, corky upon drying, pinkish (8A2) to reddish brown (8E6), velutinous, inside solid, cylindrical or attenuate downwards with bulbous base when old. Hyphal structure: hyphal system monomitic; generative hyphae with simple-septa, CB+ in slightly thick-walled hyphae, IKI-; tissues olivaceous in KOH. Context: generative hyphae hyaline, thin- to slightly thick-walled, occasionally branched, simple-septate, straight, regularly arranged, sometimes flexuous and collapsed, mostly 4-6 µm diam. Spines: generative hyphae hyaline, thin- to slightly thick-walled, moderately branched, more or less parallel along spines, frequently simple-septate, straight, 2-4 µm diam. Cystidia and cystidioles absent. Basidia clavate, thin-walled, with four sterigmata (4-6 µm long), simple-septate at base, $20-48 \times 5-8 \mu m$; basidioles similar to basidia. *Basidiospores* irregular ellipsoid, brown, thin-walled, tuberculate, CB-, IKI-, $(4.1-)4.5-6(-6.1) \times (3.2-)3.9-5.1(-6) \mu m$, Lm = 5 μm , Wm = $4.4 \,\mu m$, Q = 1.14 - 1.21 (n = 60/2); tuberculi usually isolated, sometimes in groups of two or more, then bi- to trifurcate in shape, up to 1.1 µm long.

Additional specimens (paratypes) examined: CHINA, Yunnan Province, Yulong County, on the ground in Fagaceous forest, Y. L. Wei, 23 July 2018, Wei 8290 (IFP 019378, paratype); Wei 8312 (IFP 019379, paratype); Wei 8315 (IFP 019380, paratype); Wei 8319 (IFP 019381, paratype).



Figure 8. *Hydnellum atrorubrum.* (**a**): Basidiocarps; (**b**,**c**): SEM of basidiospores; (**d**–**f**): Microscopic structures (drawn from IFP 019377); (**d**): Section of hymenophore trama with basidia; (**e**): Hyphae from pileus context; (**f**): Basidiospores. —Scale bars: (**a**) = 2.5 cm; (**b**) = $3 \mu \text{m}$; (**c**) = $1 \mu \text{m}$; (**d**,**e**) = $10 \mu \text{m}$; (**f**) = $5 \mu \text{m}$.

Notes: Phylogenetically, *Hydnellum atrorubrum* has a close relationship with *H. subsuccosum*. Morphologically, *H. subsuccosum* is similar to *H. atrorubrum* in having gregarious

to confluent basidiocarps with zonate pileal surface and lobed pileal margin, brown context, cylindrical or attenuate downwards stipe, decurrent and similar length spines, the monomitic hyphal system with uninflated and simple-septate generative hyphae, basidia of similar shape and width, as well as basidiospores of similar length. However, *H. subsuccosum* can be differentiated by scabrous to nodulose and orange-white to camel pileal surface, black spines and presence of subglobose basidiospores [27]. *H. auratile* is comparable to *H. atrorubrum* in having similar size, depressed to infundibuliform or flabelliform and concentrically zoned pileus with undulate margin, tomentose and reddish-brown stipe,

ever, *H. auratile* differs from *H. atrorubrum* by orange or orange-brown to dark red-brown pileus with entire or deeply split margin and tawny to purplish brown spines [26].

non-duplex context, thin- to slightly thick-walled and unclamped generative hyphae. How-

Hydnellum bomiense Y.H. Mu & H.S. Yuan, sp. nov. (Figure 9)

MycoBank MB839035

Etymology. Bomiense, refers to the Bomi County, where the specimens were collected. Type: CHINA, Xizang Autonomous Region, Bomi County, on the ground in Fagaceous forest, 19 July 2019, *H. S. Yuan*, Yuan 13767 (holotype IFP 019382).

Basidiocarps annual, solitary to gregarious, soft and leathery when fresh, becoming woody and light in weight upon drying; taste acrid, odor slightly fragrant when dry. Pileus infundibuliform when young, later applanate and irregularly circular with age, up to 26 mm diam and 2–4 mm thick at center. *Pileal surface* grayish yellow (4B4), brown (7E7) to dark brown (7F8), obscurely concentrically zonate, tomentose, scrupose when fresh, becoming fibrillose or glabrous when dry; margin white (5A1) when fresh, grayish orange (5B4) when dry, involute and wavy, sometimes lobed or rimose with age. Spine surface white (6A1) to brown (6E7) when fresh, light brown (6D6) to dark brown (7F8) when dry; spines up to 1.1 mm long, base up to 0.2 mm diam, conical, 4-6 per mm, more or less decurrent on stipe, without spines at pileus margin, brittle when dry. Context not duplex, up to 4 mm thick, brown (6E5), woody. Stipe central to lateral, up to 2 cm long and 0.5 cm diam, woody upon drying, grayish orange (5B5) to dark brown (7F7), rugose, solid inner, terete or attenuate downwards with bulbous base when old. Hyphal structure: hyphal system monomitic; generative hyphae with simple-septa, CB+ in slightly thick-walled hyphae, IKI-; tissues olivaceous in KOH. Context: generative hyphae hyaline, thin- to slightly thick-walled, frequently branched, simple-septate, straight, regularly arranged, sometimes flexuous and collapsed, mostly 4–6 µm diam. Spines: generative hyphae hyaline, thin-walled, occasionally branched, more or less parallel along spines, frequently simpleseptate, straight, 2-4 µm diam. Cystidia and cystidioles absent. Basidia clavate, thin-walled, with four sterigmata (1.5–3 μ m long), simple-septate at base, 15–42 \times 4–7 μ m; basidioles similar to basidia. Basidiospores irregularly ellipsoid to subglobose, brown, thin-walled, tuberculate, CB-, IKI-, $(4-)4.1-5.1(-5.2) \times (3-)3.3-4.5(-4.8) \mu m$, Lm = 4.7 μm , Wm = 4 μm , Q = 1.18-1.21 (n = 60/2); tuberculi usually isolated, sometimes in groups of two or more, then bi- to trifurcate in shape, up to 1 µm long.

Additional specimen (paratype) examined: CHINA, Xizang Autonomous Region, Bomi County, on the ground with moss in Fagaceous forest, 19 July 2019, *H. S. Yuan*, Yuan 13759 (IFP 019383, paratype).



Figure 9. *Hydnellum bomiense.* (**a**,**b**): Basidiocarps; (**c**,**d**): SEM of basidiospores; (**e**–**g**): Microscopic structures (drawn from IFP 019382); (**e**): Section of hymenophore trama with basidia; (**f**): Hyphae from pileus context; (**g**): Basidiospores. —Scale bars: (**a**,**b**) = 1 cm; (**c**) = 3 μ m; (**d**) = 1 μ m; (**e**,**f**) = 10 μ m; (**g**) = 5 μ m.

Notes: This species clustered with two samples from Estonia and Costa Rica, and formed an independent clade (Figure S1). *Hydnellum bomiense* and *H. dianthifolium* are closely related based on nucleotide sequence analyses and possess common morphological features: separate to coalescing or grouped basidiocarps with decurrent spines, cylindrical stipe, absence of clamp-connections and brown, tuberculate and irregularly ellipsoid to

subglobose basidiospores of similar size with isolated to bifurcate tuberculi. However, *H. dianthifolium* differs from *H. bomiense* by having slender, turbinate and coralloid basidiocarps that split radially to form erect, coralloid or flower-shaped lobed pilei, not perceptibly zoned context and thick-walled and encrusted context hyphae [36]. Meanwhile, *H. concrescens* and *H. scrobiculatum* are also in a big clade with *H. bomiense* in Figure 1, and concentrically zonate pileal surface is their common characteristic. However, *H. concrescens* differs by quite larger pileus (up to 120 mm vs. up to 26 mm in *H. bomiense*), duplex context and longer basidia sterigmata (3–4 μ m vs. 1.5–3 μ m in *H. bomiense*) [27,34]. *H. scrobiculatum* differs in subcolliculose to scrobiculate pileal surface and longer spines (up to 3 mm vs. 1.1 mm in *H. bomiense*) [27,34].

Hydnellum rubidofuscum Y.H. Mu & H.S. Yuan, **sp. nov.** (Figure 10) MycoBank MB839041

Etymology. Rubidofuscum (Latin), refers to the reddish brown pileal surface.

Type: CHINA, Liaoning Province, Xinbin County, Gangshan Nature Reserve, on the ground in *Quercus* forest, 12 August 2020, *H. S. Yuan*, Yuan 14561 (holotype IFP 019408).

Basidiocarps annual, solitary to gregarious or multiple pilei overlapping and fused to form a compound cluster, soft and leathery when fresh, becoming woody and light in weight upon drying; taste mild, odor slightly fragrant when dry. *Pileus* applanate to infundibuliform when young, later depressed to flabelliform or irregularly circular with age, up to 70 mm diam and 4–10 mm thick at center. *Pileal surface* reddish brown (8E8), obscurely concentrically zonate, glabrous to scrupose when fresh, becoming fibrillose to virgate when dry; margin white (6A1) to orange-white (6A2) when fresh, brownish orange (6C4) when dry, even, sometimes lobed with age. Spine surface gravish brown (8D3) to reddish brown (8E7) when fresh, brown (6E6) to dark brown (6F7) when dry; spines up to 3 mm long, base up to 0.2 mm diam, conical, 4–6 per mm, strongly decurrent on stipe, without spines at pileus margin, brittle when dry. Context not duplex, up to 10 mm thick, brown (6E6), woody. Stipe central to lateral, up to 3.5 cm long and 2 cm diam, sometimes connate, leathery when fresh, woody upon drying, light brown (7D6) to brown (7E6), pubescent, solid inner, cylindrical to flat or attenuate downwards with bulbous base when old. Hyphal structure: hyphal system monomitic; generative hyphae with simple-septa, CB+ in slightly thick-walled hyphae, IKI-; tissues olivaceous in KOH. Context: generative hyphae hyaline, thin- to slightly thick-walled, occasionally branched, simple-septate, straight, regularly arranged, sometimes flexuous and collapsed, mostly 3-6 µm diam. Spines: generative hyphae hyaline, thin-walled, occasionally branched, more or less parallel along spines, frequently simple-septate, straight, 2–3 µm diam. Cystidia and cystidioles absent. Basidia clavate, thin-walled, with four sterigmata (1-4 µm long), simple-septate at base, 14–37 \times 4–6 μ m; basidioles similar to basidia. *Basidiospores* irregularly ellipsoid to subglobose, brown, thin-walled, tuberculate, CB-, IKI-, $(4-)4.1-5(-5.1) \times (3.8-)3.9-4.6(-4.8) \mu m$, $Lm = 4.6 \mu m$, $Wm = 4.1 \mu m$, Q = 1.11-1.12 (n = 60/2); tuberculi usually isolated, sometimes in groups of two or more, then bi- to trifurcate in shape, up to 1 µm long.

Additional specimens (paratypes) examined: CHINA, Liaoning Province, Xinbin County, Gangshan Nature Reserve, on the ground in *Quercus* forest, 12 August 2020, *H. S. Yuan*, Yuan 14559 (IFP 019409, paratype); Yuan 14560 (IFP 019410, paratype); Yuan 14563 (IFP 019411, paratype); 26 August 2020, *H. S. Yuan*, Yuan 14586 (IFP 019412, paratype); Yuan 14587 (IFP 019413, paratype); 12 September 2020, *H. S. Yuan*, Yuan 14792 (IFP 019414, paratype); Yuan 14794 (IFP 019415, paratype); Yuan 14800 (IFP 019416, paratype); Benxi County, Guanmenshan National Forest Park, on the ground in *Quercus* forest, 29 August 2020, *H. S. Yuan*, Yuan 14654 (IFP 019417, paratype).



Figure 10. *Hydnellum rubidofuscum.* (a): Basidiocarps; (b,c): SEM of basidiospores; (d–f): Microscopic structures (drawn from IFP 019408); (d): Section of hymenophore trama with basidia; (e): Hyphae from pileus context; (f): Basidiospores. —Scale bars: (a) = 1 cm; (b) = 3 μ m; (c) = 1 μ m; (d,e) = 10 μ m; (f) = 5 μ m.

Notes: Phylogenetically, *Hydnellum rubidofuscum* is closely related to *H. bomiense* and *H. dianthifolium* (Figure 1). Morphologically, infundibuliform pileus when young,

concentrically zonate pileal surface and irregularly ellipsoid to subglobose basidiospores are their common features. However, *H. bomiense* can be distinguished by smaller pileus (up to 26 mm vs. up to 70 mm in *H. rubidofuscum*), yellow to dark brown pileal surface and shorter spines (up to 1.1 mm vs. up to 3 mm in *H. rubidofuscum*). *H. dianthifolium* differs by subpubescent pileal surface, reddish-brown to vinaceous-brown context, thick-walled and rarely branched hyphae in the context and context tissue turning blue-green in KOH [36]. The reddish brown pileal surface is very similar to that of *H. scrobiculatum*. Furthermore, *H. scrobiculatum* also has a single to gregarious or concrescent basidiocarp with applanate to depressed or infundibuliform pileus, obscurely concentrically zonate pileal surface, simple or connate, central to eccentric and velutinous stipe, reddish brown and decurrent spines and the monomitic hyphal system. However, the major differences are that *H. scrobiculatum* has a fungoid or no odor, a scrobiculate and rugulose pileal surface, duplex and zonate context and longer basidiospores (5.4–6.4 µm vs. 4.1–5 µm in *H. rubidofuscum*) [26,27,34].

Hydnellum squamulosum Y.H. Mu & H.S. Yuan, sp. nov. (Figure 11)

MycoBank MB839042

Etymology. Squamulosum (Latin) refers to the minutely scaly pileal surface.

Holotype. CHINA, Xizang Autonomous Region, Bomi County, on the ground in *Picea* mixed forest, 17 July 2019, *H. S. Yuan*, Yuan 13615 (holotype IFP 019418).

Basidiocarps annual, solitary to gregarious or coalescent to form complex pileus, soft and leathery when fresh, becoming corky and light in weight upon drying; taste none, odor none when dry. Pileus circular when young, circular or semicircular with age, applanate, up to 35 mm diam and 4-8 mm thick at center. Pileal surface pastel red (7A4) to dark Magenta (13F7), zonate, floccose to woolly, squamulose when fresh, becoming fibrillose and scrobiculate when dry; margin white (7A1) when fresh, grayish orange (5B3) when dry, involute and wavy, sometimes lobed with age. Spine surface pale red (7A3) to reddish brown (8E8) when fresh, grayish orange (5B4) to dark brown (8F7) when dry; spines up to 2 mm long, base up to 0.2 mm diam, conical, 3–6 per mm, decurrent on stipe, without spines at pileus margin, brittle when dry. Context not duplex, up to 9 mm thick, reddish brown (8E6), soft corky. Stipe central to lateral, up to 4 cm long and 1 cm diam, sometimes connate, leathery or freshy when fresh, soft corky upon drying, pale red (11A3), tomentose, solid inner, context with a dark zone present at centre, terete to flat or attenuate downwards with bulbous base when old. Hyphal structure: hyphal system monomitic; generative hyphae with simple-septa, occasionally encrusted, CB+ in slightly thick-walled hyphae, IKI-; tissues olivaceous in KOH. Context: generative hyphae hyaline, thin- to slightly thickwalled, moderately branched, simple-septate, straight, regularly arranged, sometimes flexuous and collapsed, mostly 4–5 µm diam. Spines: generative hyphae hyaline, thinwalled, moderately branched, more or less parallel along spines, frequently simple-septate, straight, 2-4 µm diam. Cystidia and cystidioles absent. Basidia clavate, thin-walled, with four sterigmata (2–3 μ m long), simple-septate at base, 8–38 \times 4–6 μ m; basidioles similar to basidia. Basidiospores irregularly ellipsoid to globose, brown, thin-walled, tuberculate, CB-, IKI-, $(4-)4.1-5(-5.1) \times (3.2-)3.3-4.1(-4.2) \mu m$, Lm = 4.4 μm , Wm = 3.8 μm , Q = 1.14-1.16 (n = 60/2); tuberculi usually isolated, sometimes in groups of two or more, then bi- to trifurcate in shape, up to $1 \mu m \log$.

Additional specimens (paratypes) examined: CHINA, Xizang Autonomous Region, Bomi County, on the ground in *Picea* mixed forest, 17 July 2019, *H. S. Yuan*, Yuan 13617 (IFP 019419, paratype); Yuan 13625 (IFP 019420, paratype); Yuan 13626 (IFP 019421, paratype); Yuan 13627 (IFP 019422, paratype); on the ground with moss in *Picea* mixed forest, 19 July 2019, *H. S. Yuan*, Yuan 13743 (IFP 019423, paratype).



Figure 11. *Hydnellum squamulosum.* (a): Basidiocarps; (b,c): SEM of basidiospores; (d–f): Microscopic structures (drawn from IFP 019418); (d): Section of hymenophore trama with basidia; (e): Hyphae from pileus context; (f): Basidiospores. —Scale bars: (a) = 1 cm; (b) = 3 μ m; (c) = 1 μ m; (d,e) = 10 μ m; (f) = 5 μ m.

Notes: *Hydnellum squamulosum* and *H. concrescens* are closely related in the phylogenetic tree and share similar morphological and anatomical characteristics: a solitary to

gregarious or coalescent basidiocarp with fibrillose, squamulose and zonate pileal surface, irregularly lobed margin, decurrent and reddish-brown spines, not duplex context in the pileus, zonate context in the stipe, context tissue becoming olivaceous in KOH, and tuberculate basidiospores. However, *H. concrescens* can be differentiated by depressed or infundibuliform basidiocarps, reddish white to dark brown pileal margin, larger pileus (up to 120 mm vs. 35 mm in *H. squamulosum*), longer basidia sterigmata (up to 5 µm vs. up to 3 µm in *H. squamulosum*) and larger basidiospores ($5-6 \times 4-5 µm vs. 4.1-5 \times 3.3-4.1 µm$ in *squamulosum*) [2,27,34]. *H. fraudulentum* is similar to *H. squamulosum* in having a squamulose-fibrillose pileal surface, cylindrical or connate stipe, context tissue olivaceous in KOH, thinto slightly thick-walled and unclamped hyphae in the context, basidia of similar shape, and brown and tuberculate basidiospores. However, it differs from *H. squamulosum* in having depressed, azonate and yellow-brown to dark brown pilei, purplish brown and slightly longer spines (up to 2.5 mm vs. 2 mm in *H. squamulosum*), wider basidia (6-7 µm vs. 4-6 µm in *H. squamulosum*) with longer sterigmata (3.6-4.5 µm vs. 2-3 µm in*H. squamulosum*) [26].

Hydnellum sulcatum Y.H. Mu & H.S. Yuan, sp. nov. (Figure 12)

MycoBank MB839043;

Etymology. Sulcatum (Latin), refers to the often grooved pileal surface.

Type: CHINA, Liaoning Province, Benxi County, Guanmenshan National Forest Park, on the ground of *Quercus* forest, 29 August 2020, *H. S. Yuan*, Yuan 14649 (holotype IFP 019424).

Basidiocarps annual, solitary to gregarious or multiple pilei overlapping and fused to form a compound cluster, soft and leathery when fresh, becoming woody and light in weight upon drying; taste mild, odor slightly fragrant when dry. Pileus subinfundibuliform when young, later applanate to flabelliform or circular with age, up to 65 mm diam and 3-6 mm thick at center. Pileal surface dark brown (9F4), obscurely concentrically zonate, often grooved, scabrous to fibrous when fresh, becoming fibrillose, rugose when dry; margin white (6A1) when fresh, light brown (6D5) when dry, even, sometimes lobed. Spine surface brown (7E7) when fresh, brown (6E5) to dark brown (7F5) when dry; spines up to 1.5 mm long, base up to 0.1 mm diam, conical, 5–7 per mm, more or less decurrent on stipe, without spines at pileus margin, brittle when dry. *Context* not duplex, up to 6 mm thick, brown (7E6), woody. Stipe lateral, up to 2 cm long and 1.5 cm diam, sometimes connate, leathery when fresh, woody upon drying, brown (7E5), pubescent, inside solid, cylindrical to flat or attenuate downwards with bulbous base when old. Hyphal structure: hyphal system monomitic; generative hyphae with simple-septa, CB+ in slightly thick-walled hyphae, IKI-; tissues olivaceous in KOH. Context: generative hyphae hyaline, thin-walled, moderately branched, simple-septate, straight, regularly arranged, sometimes flexuous and collapsed, mostly 4–6 µm diam. Spines: generative hyphae hyaline, thin to slightly thick-walled, moderately branched, more or less parallel along spines, often simple-septate, straight, 2-3 µm diam. Cystidia and cystidioles absent. Basidia clavate, thin-walled, with four sterigmata (2–3 μ m long), simple-septate at base, 20–30 \times 4–8 μ m; basidioles similar to basidia. Basidiospores irregular ellipsoid to subglobose, brown, thin-walled, tuberculate, CB-, IKI-, (4–)4.1–5.8(–5.9) \times (3.9–)4–4.6(–4.8) μ m, Lm = 4.8 μ m, Wm = 4.3 μ m, Q = 1.14–1.19 (n = 60/2); tuberculi usually isolated, sometimes in groups of two or more, then bi- to trifurcate in shape, up to 0.9 µm long.

Additional specimens (paratypes) examined: CHINA, Liaoning Province, Kuandian County, Baishilazi National Nature Reserve, on the ground of *Quercus* forest, 8 August 2020, *H. S. Yuan*, Yuan 14521 (IFP 019425, paratype); Benxi County, Guanmenshan National Forest Park, on the ground of *Quercus* forest, 29 August 2020, *H. S. Yuan*, Yuan 14638 (IFP 019426, paratype); Yuan 14658 (IFP 019427, paratype); Yuan 14660 (IFP 019428, paratype).



Figure 12. *Hydnellum sulcatum.* (**a**): Basidiocarps; (**b**,**c**): SEM of basidiospores; (**d**–**f**): Microscopic structures (drawn from IFP 019424); (**d**): Section of hymenophore trama with basidia; (**e**): Hyphae from pileus context; (**f**): Basidiospores. —Scale bars: (**a**) = 2 cm; (**b**) = 3 μ m; (**c**) = 1 μ m; (**d**,**e**) = 10 μ m; (**f**) = 5 μ m.

Notes: *Hydnellum parvum* has a close phylogenetic relationship with *H. sulcatum*. The former species resembles the latter by compound fused pilei and rugulose pileal surface.

However, the latter has a thinner stipe $(0.7 \times 0.2 \text{ cm vs. } 2 \times 1.5 \text{ cm in } H. sulcatum)$, slightly longer spines (up to 2 mm vs. 1.5 mm in *H. sulcatum*) and shorter basidiospores (3–4 µm vs. 4.1–5.8 µm in *H. sulcatum*) [27]. *H. subsuccosum* resembles *H. sulcatum* by concentrically zonate pileus with similar size, context hyphae of similar width, the absence of clampconnections and brown and subglobose basidiospores. However, *H. subsuccosum* differs by orange white to camel and nodulose or pitted pileal surface, longer spines (up to 3 mm vs. 1.5 mm in *H. sulcatum*), blue green to dark brown or black context tissue in KOH and longer basidia sterigmata (3–4 µm vs. 2–3 µm in *H. sulcatum*) [27]. *H. atrorubrum* is similar to *H. sulcatum* in having white to brown and even pileal margin and zonate pileal surface, olivaceous context tissues in KOH and basidiospores of similar shape with isolated or grouped tuberculi. However, *H. atrorubrum* can be differentiated by a flocculose to fibrillose or glabrescent pileal surface, longer spines (up to 3.5 mm vs. 1.5 mm in *H. sulcatum*) and basidia sterigmata (4–6 µm vs. 2–3 µm in *H. sulcatum*) and slightly longer tuberculi (up to 1.1 µm vs. 0.9 µm in *H. sulcatum*) of basidiospores.

Hydnellum yunnanense Y.H. Mu, X.H. Wang & H.S. Yuan, **sp. nov.** (Figure 13) MycoBank MB839044

Etymology. Yunnanense, refers to the Yunnan Province, where the specimens were collected.

Type: CHINA, Yunnan Province, Nanjian County, Lingbaoshan National Forest Park, on the ground, 19 September 2019, *H. S. Yuan*, Yuan 14386 (holotype IFP 019429).

Basidiocarps annual, solitary to gregarious, soft and leathery when fresh, becoming woody and light in weight upon drying; taste mild, odor slightly fragrant when dry. Pileus subinfundibuliform when young, later flabelliform with age, up to 21 mm diam and 3-5 mm thick at center. Pileal surface gravish red (10D6) to dark brown (9F8), obscurely concentrically zonate, velutinate to tomentose when fresh, becoming rugulose to glabrescent when dry; margin white (6A1) when fresh, light brown (6D4) when dry, even, sometimes eroded with age. Spine surface white (10A1) to gravish red (106) when fresh, pale orange (5A3) to brown (6E7) when dry; spines up to 1.5 mm long, base up to 0.1 mm diam, conical, 5-8 per mm, more or less decurrent on stipe, without spines at pileus margin, brittle when dry. Context not duplex, up to 5 mm thick, reddish brown (8E4), woody. Stipe central to lateral, up to 4 cm long and 0.7 cm diam, sometimes connate, leathery when fresh, woody upon drying, brown (7E6), tomentose, solid inner, context with a dark line present at centre, cylindrical to attenuate downwards or broadening below with bulbous base when old. Hyphal structure: hyphal system monomitic; generative hyphae with simple-septa, CB+ in slightly thickwalled hyphae, IKI-; tissues olivaceous in KOH. Context: generative hyphae hyaline, thinto slightly thick-walled, moderately branched, simple-septate, straight, regularly arranged, sometimes flexuous and collapsed, mostly 3–5 µm diam. Spines: generative hyphae hyaline, thin- to slightly thick-walled, frequently branched, more or less parallel along spines, often simple-septate, straight, 2–5 µm diam. Cystidia and cystidioles absent. Basidia clavate, thin-walled, with four sterigmata (2–4 μ m long), simple-septate at base, 13–28 \times 4–7 μ m; basidioles similar to basidia. Basidiospores irregularly ellipsoid to subglobose, brown, thinwalled, tuberculate, CB-, IKI-, (4.1-)4.2-5.1(-5.3) \times (3.4-)3.5-4.5(-5) μ m, Lm = 4.7 μ m, Wm = 4 μ m, Q = 1.17–1.18 (n = 60/2); tuberculi usually isolated, sometimes in groups of two or more, then bi- to trifurcate in shape, up to 1.2 μm long.

Additional specimens (paratypes) examined: CHINA, Yunnan Province, Nanjian County, Lingbaoshan National Forest Park, on the ground, 19 September 2019, *H. S. Yuan*, Yuan 14396 (IFP 019430, paratype); Maguan County, Dalishu Township, Adushangba Village, on the ground, 7 August 2017, *S. F. Shi*, Shi 212 (IFP 019431, paratype).



Figure 13. *Hydnellum yunnanense.* (**a**,**b**): Basidiocarps; (**c**,**d**): SEM of basidiospores; (**e**–**g**): Microscopic structures (drawn from IFP 019429); (**e**): Section of hymenophore trama with basidia; (**f**): Hyphae from pileus context; (**g**): Basidiospores. —Scale bars: (**a**,**b**) = 1 cm; (**c**) = 3 μ m; (**d**) = 1 μ m; (**e**,**f**) = 10 μ m; g = 5 μ m.

Notes: The phylogenetic analyses support that *Hydnellum yunnanense* is sister to *H. sulcatum* (Figure 1). Morphologically, *H. sulcatum* resembles *H. yunnanense* in having an annual, solitary to gregarious basidiocarp with subinfundibuliform to flabelliform pileus with equal-length spines, a brown, pubescent, cylindrical stipe, olivaceous context tissue in KOH and basidiospores of similar shape. However, it differs from *H. yunnanense*

in having a broader pileus (up to 65 mm vs. 21 mm in *H. yunnanense*) with scabrous to squamulose pileal surface and light brown pileal margin and shorter tuberculi (up to 0.9 μ m vs. 1.2 μ m in *H. yunnanense*). *H. rubidofuscum* resembles *H. yunnanense* by infundibuliform to flabelliform and concentrically zonate pileus, not duplex and woody context, thin- to slightly thick-walled and simple-septate hyphae in the context, basidia of similar width and basidiospores of similar shape and size. However, *H. rubidofuscum* differs by larger pileus (up to 70 mm vs. up to 21 mm in *H. yunnanense*) with reddish brown and scrupose, fibrillose to virgate pileal surface, grayish brown to reddish brown and longer spines (up to 3 mm vs. up to 1.5 mm in *H. yunnanense*) and thin-walled generative hyphae in the spines.

Hydnellum sp 3

Basidiocarps annual, solitary. *Pileus* applanate, flabelliform. *Pileal surface* brown (7E7) to dark brown (7F7), concentrically zonate and pubescent. *Spines* reddish brown (9E6) and more or less decurrent, up to 1 mm long. *Stipe* lateral, cylindrical and slender. *Hyphal system* monomitic, generative hyphae simple-septa. *Basidia* clavate, with four sterigmata. *Basidiospores* irregularly subglobose, tuberculate, $(4.8-)4.9-5.2(-5.3) \times (4-)4.1-4.8(-5) \mu m$.

Specimen examined: CHINA, Yunnan Province, Nanjian County, Lingbaoshan National Forest Park, on the ground of angiosperm forest, 19 September 2019, *H. S. Yuan*, Yuan 14388 (IFP 019438).

Hydnellum sp 4

Basidiocarps annual, solitary to gregarious. *Pileus* irregularly flabelliform. *Pileal sur-face* grayish yellow (4B3) to yellowish brown (5E8), obscurely concentrically zonate and glabrescent. *Spines* yellowish brown (5D5) and more or less decurrent, up to 2 mm long. *Stipe* lateral and cylindrical. *Hyphal system* monomitic, generative hyphae simple-septa. *Basidia* clavate, with four sterigmata. *Basidiospores* irregularly subglobose, tuberculate, $(4.1-)4.2-5.3(-6) \times (4-)4.1-4.3(-4.8) \mu m$.

Specimen examined: CHINA, Yunnan Province, Maguan County, Dalishu Township, on the ground in Fagaceous forest, 14 October 2017, *J. Wang*, Wang 295 (IFP 019439).

Hydnellum sp 5

Basidiocarps annual, solitary. *Pileus* irregularly circular. *Pileal surface* zonate, velutinous and strigose with lobed margin. *Spines* decurrent, up to 1 mm long. *Stipe* lateral, cylindrical and broadened below with bulbous base when old. *Hyphal system* monomitic, generative hyphae simple-septa. *Basidia* clavate, with four sterigmata. *Basidiospores* irregularly ellipsoid to subglobose, tuberculate, $(4-)4.1-5.5(-5.6) \times (3-)3.1-4.7(-5.2) \mu m$.

Specimen examined: CHINA, Liaoning Province, Xinbin County, Gangshan Nature Reserve, in the angiosperm forest dominated by *Quercus liaotungensis*, 26 August 2020, *H. S. Yuan*, Yuan 14594 (IFP 019440).

Other *Hydnellum* species

Hydnellum complicatum, H. cumulatum, H. diabolus, H. geogenium, H. lundellii, H. martioflavus, **H. peckii**, H. regium, H. versipelle

Hydnellum peckii Banker, in Peck, Bull. N.Y. St. Mus. 157: 28 (1912) (1911)

Basidiocarps single to concrescent. *Pileus* turbinate or elliptical, planar to subdepressed. *Pileal surface* white (6A1) to light orange (6A4), colliculose, rarely scrobiculate and glabrous. *Spines* brownish orange (7C4), decurrent, up to 3 mm long. *Stipe* central and terete. *Hyphal system* monomitic; generative hyphae mostly with clamp-connections, minority of simplesepta. *Basidia* clavate, with simple-septate at base and four sterigmata. *Basidiospores* irregularly subglobose, tuberculate, (4.1–)4.2–5.1(–5.3) × (3.8–)3.9–4.4(–4.6) µm.

Specimens examined. CHINA, Xizang Autonomous Region, Bomi County, on the ground in *Pinus* mixed forest, 19 July 2019, *H. S. Yuan*, Yuan 13708 (IFP 0193433); Yuan 13720 (IFP 019434).

Notes: The studied samples were found in high altitude *Pinus* mixed forest. These two specimens clustered with two other samples (SSvantesson328 and ELarsson174-14) of *Hydnellum peckii* from Europe with full support (100% in ML and 1.00 BPP) (Figure 1). The ITS+LSU sequence identity between the specimens from China and Europe was 0.99 in

Bioedit pairwise alignment. In morphology, the characters of our specimens overlap with those of *H. peckii* [1,27]. Therefore, we introduce *H. peckii* as a new record to China.

Key to species of Hydnellum from China

1. Basidiocarps fleshy	2
$1. \rightarrow$ Basidiocarps woody	4
2. Pileal surface not scaled	H. coactum
$2. \rightarrow$ Pileal surface scaled	3
3. Pileal surface with ascend squama	H. grosselepidotum
$3. \rightarrow$ Pileal surface with appressed squama	H. lidongensis
4. Context tissue olivaceous in KOH	5
$4. \rightarrow \text{Context tissue blue-green in KOH}$	H. peckii
5. Hyphae with clamp-connections in the context	6
5. \rightarrow Hyphae without clamp-connections in the context and spines	8
6. Basidiocarps with dark violet spines underneath pileus	H. atrospinosum
$6. \rightarrow$ Basidiocarps with different colored spines underneath pileus	7
7. Pileal surface fibrillose, rugose when dry, spines up to 1.5 mm long	H. fibulatum
$7. \rightarrow$ Pileal surface pitted, colliculose when dry, spines up to 6 mm long	H. caeruleum
8. Inflated hyphae present from the context	9
$8. \rightarrow$ Inflated hyphae absent in the context	10
9. Generative hyphae mostly inflated in the context, pileal surface scrobiculate when dry	H. inflatum
$9. \rightarrow$ Generative hyphae occasionally inflated in the context, pileal surface granulose when dry	H. granulosum
10. Stipe thin, rhizomorphs-like	Hydnellum sp 1
$10. \rightarrow$ Stipe cylindrical to flattened	11
11. Pileal surface colored brownish orange to brownish red	H. brunneorubrum
$11. \rightarrow$ Pileal surface differently colored	12
12. Pileal margin involute and wavy, sometimes lobed or rimose	13
$12. \rightarrow$ Pileal margin even or effused, sometimes lobed or eroded	14
13. Pileal surface glabrescent	<i>Hydnellum</i> sp 4
$13. \rightarrow$ Pileal surface not glabrescent	15
14. Pileal surface azonate and spongy	16
$14. \rightarrow$ Pileal surface obsurely concentrically zonate to zonate and not spongy	17
15. Pileal surface floccose to squamulose when fresh, context corky	H. squamulosum
$15. \rightarrow$ Pileal surface tomentose and scrupose when fresh, context woody	H. bomiense
16. Basidiocarps single to gregarious and stipe single and long	H. spongiosipes
$16. \rightarrow$ Basidiocarps coalescent and stipe connate and short	Hydnellum sp 2
17. Stipe context with a dark line at centre	18
$17. \rightarrow $ Stipe context without a dark line at centre	19
18. Pileus and spines grayish red	H. yunnanense
$18. \rightarrow$ Pileus brown and spines reddish brown	Hydnellum sp 3
19. Spines up to 3 or 3.5 mm long	20
$19. \rightarrow$ Spines up to 1 or 1.5 mm long	21
20. Pileus light brown to dark ruby	H. atrorubrum
$20. \rightarrow$ Pileus reddish brown	H. rubidofuscum
21. Basidiocarps gregarious or multiple pilei overlapping and pileal surface often grooved,	II autorium
scabrous to fibrous	H. sulcatum
$21. \rightarrow$ Basidiocarps solitary and pileal surface velutinous and strigose	<i>Hydnellum</i> sp 5
	с I

5. Discussion

Baird et al. [27] constructed a phylogenetic tree of Bankeraceae using ITS sequences of specimens from the temperate southeastern United States, and suggested that neither *Hydnellum* nor *Sarcodon* were monophyletic. In the current study, phylogenetic analyses using four loci (nLSU + ITS + SSU + RPB2) of *Hydnellum* and *Sarcodon* were carried out using sequences from Europe, Asia and America. Our results also verify that *Sarcodon* is paraphyletic; with regard to *Hydnellum*, some species of *Sarcodon* nesting among *Hydnellum* species. Larsson et al. [31] provided phylogenetic analyses on *Hydnellum* and *Sarcodon* based on ITS and LSU sequences, confirming that *Sarcodon* moved to *Hydnellum*. Morphologically,

they proposed to use the size of the basidiospores to separate the two genera, delimiting *Hydnellum* and *Sarcodon* species with basidiospore lengths in the ranges of 4.45–6.95 μ m and 7.4–9 μ m, respectively. These results indicate that the traditional classification system based on the consistency of *Sarcodon* (fleshy) and *Hydnellum* (woody) did not conform to the monophyletic concept of the two genera.

Based on microscopical hyphal structure species of *Hydnellum* and *Sarcodon* can be divided into five groups, as shown in Table 3: (I) Context of the pileus and the spines composed of simple-septate hyphae (includes *H. aurantiacum*, *H. auratile* and 49 other taxa); (II) Pileus composed mostly of simple-septate hyphae with occasional clamp-connections and spinal trama composed of simple-septate hyphae (includes *H. caeruleum*, *H. ferrugipes* and *H. fibulatum*); (III) Both pileus and the spines composed of predominantly clamped hyphae with occasional simple-septa (includes *H. peckii* and *H. versipelle*); (IV) Pileus composed of only hyphae with clamp-connections and spine with only simple-septate hyphae (includes *H. diabolus*); (V) Both pileus and the spines composed of clamped hyphae (includes *H. diabolus*); (V) Both pileus and the spines composed of clamped hyphae (includes *H. atrospinosum*, *H. cyanopodium*, *H. geogenium*, *H. scleropodium*, *H. suaveolens*, *S. aspratus*, *S. imbricatus*, *S. leucopus*, *S. quercinofibulatus*, *S. scabripes* and *S. squamosus*). One species, *H. regium*, has not been classified into these five groups because the information is not available from the original description [33].

In the phylogenetic tree, ten subgenera with moderate to high support in *Hydnellum* have been distinguished (Figure 1), and each subgenus possesses distinctive morphological characteristics. Group I can be categorized into seven subgenera. The subgenus Croceum has an orange basidiocarps and includes H. aurantiacum, H. auratile, H. brunneorubrum, H. *chrysinum* and *H. earlianum*; the subgenus *Inflatum* has the appearance of inflated hypha in the context of the pileus and includes H. cristatum, H. granulosum, H. inflatum, H. mirabile and *H. piperatum*; the subgenus *Rhizomorphum* has rhizomorphs-like stipe and includes *H.* gracilipes and Hydnellum sp 1; the subgenus Scabrosum has pileal surface with scabrosity and includes H. amygdaliolens, H. coactum, H. fagiscabrosum, H. fennicum, H. grosselepidotum, H. illudens, H. lepidum, H. lidongensis, H. nemorosum, H. scabrosellum, H. scabrosum and H. underwoodii; the subgenus Spongiosum corresponds to a spongy pileal surface and includes H. ferrugineum, H. pineticola, H. spongiosipes and Hydnellum sp 2; the subgenus Violaceum corresponds to the violaceous basidiocarps and includes H. fuligineoviolaceum, H. fuscoindicum, H. glaucopus, H. joeides and H. roseoviolaceum; the subgenus Zonatum has a concentrically zonate pileal surface and includes H. atrorubrum, H. bomiense, H. concrescens, H. dianthifolium, H. parvum, H. rubidofuscum, H. scrobiculatum, H. squamulosum, H. subsuccosum, H. sulcatum, H. yunnanense, Hydnellum sp 3, Hydnellum sp 4 and Hydnellum sp 5. Group II corresponds to the subgenus Subindufibulatum, having occasionally clamped hyphae in the context of the pileus, and includes *H. caeruleum*, *H. ferrugipes* and *H. fibulatum*. Group V corresponds to all hyphae clamped in the pileus and the spines, incorporating two subgenera of Hydnellum and six Sarcodon species; the subgenus Hydnellum has dark spines and includes *H. atrospinosum* and *H. suaveolens*; the subgenus *Caesispinosum* has blue spines and includes H. cyanopodium and H. scleropodium; H. geogenium is morphologically related to other species in Group V, but is distantly related in the phylogenetic tree; Sarcodon clade S. aspratus, S. imbricatus, S. leucopus, S. quercinofibulatus, S. scabripes and S. squamosus have the characteristics of long spores compared with the *Hydnellum* species. Therefore, the classification system using hyphal structure type and phylogenetic subgenera can fix the positions for most species in *Hydnellum* and *Sarcodon*, except for some species in Groups III, IV and those without phylogenetic support.

The specimens involved in this study were collected from the northeast, northwest and southwest regions of China, where industrial pollution is relatively low and vegetation is relatively abundant. The forests are primarily dominated by Pinaceae and Fagaceae trees such as *Pinus* spp., *Picea* spp., *Quercus* spp., *Lithocarpus* spp. and a small portion of other tree families. Thus, we speculated that these species may form an ectomycorrhizal association with Pinaceae and Fagaceae host trees. The species diversity and basidiocarps richness of stipitate hydnoid fungi represented by *Hydnellum* and *Sarcodon* species have shown a declining trend across Europe and some regions of North America during the 1970s to 2000s [6,55–57]; the phenomenon is most probably caused by forest management, air pollutants, forest soil acidification, nitrogen deposition and forest succession, among other causes [19,58–61]. Many stipitate hydnoid fungi have been included in national Red Lists in Europe [27,62–64]. With the rapid industrialization in China over the past four decades, a significant decline of basidiocarps has also been observed during the course of our field investigation. The identification and description of stipitate hydnoid fungi in this paper will contribute to the understanding of species diversity and provide baseline data for the evaluation and protection of these fungi in China.

Table 3. Hyphal-septa type observations in the context of the pileus and the spine trama in the species of *Hydnellum* and *Sarcodon*.

Group	Subgenus	Species	Pileus	Spines	References
		Hydnellum aurantiacum	simple-septa	simple-septa	Maas Geesteranus 1975 [1]
	6	H. auratile	simple-septa	simple-septa	Maas Geesteranus 1971 [26]
	Croceum	H. brunneorubrum	simple-septa	simple-septa	In this study
		H. chrysinum	simple-septa	simple-septa	Baird 1986 [51]
		H. earlianum	simple-septa	simple-septa	Baird et al. 2013 [27]
		H. cristatum	simple-septa	simple-septa	Baird et al. 2013 [27]
		H. granulosum	simple-septa	simple-septa	In this study
	Inflatum	H. inflatum	simple-septa	simple-septa	In this study
		H. piperatum	simple-septa	simple-septa	Baird et al. 2013 [27]
		H. mirabile	simple-septa	simple-septa	Maas Geesteranus 1975 [1]
	Dhizomomlum	H. gracilipes	simple-septa	simple-septa	Koljalg & Renvall 2000 [35]
	Кпіготогрпит	Hydnellum sp 1	simple-septa	simple-septa	In this study
	Scabrosum	H. amygdaliolens	simple-septa	simple-septa	Rubio Casas et al. 2011 [52]
		H. coactum	simple-septa	simple-septa	Mu et al. 2020 [38]
_		H. fagiscabrosum	simple-septa	simple-septa	Nitare et al. 2021 [53]
I		H. fennicum	simple-septa	simple-septa	Hahn et al. 2018 [54]
		H. grosselepidotum	simple-septa	simple-septa	Mu et al. 2020 [38]
		H. illudens	simple-septa	simple-septa	Nitare et al. 2021 [53]
		H. lepidum	simple-septa	simple-septa	Maas Geesteranus 1975 [1]
		H. lidongensis	simple-septa	simple-septa	Mu et al. 2020 [38]
		H. nemorosum	simple-septa	simple-septa	Nitare et al. 2021 [53]
		H. scabrosellum	simple-septa	simple-septa	Nitare et al. 2021 [53]
		H. scabrosum	simple-septa	simple-septa	Baird et al. 2013 [27]
		H. underwoodii	simple-septa	simple-septa	Baird et al. 2013 [27]
		H. ferrugineum	simple-septa	simple-septa	Baird et al. 2013 [27]
	Snongiosum	H. pineticola	simple-septa	simple-septa	Baird et al. 2013 [27]
	Spongiosum	H. spongiosipes	simple-septa	simple-septa	Baird et al. 2013 [27]
		Hydnellum sp 2	simple-septa	simple-septa	In this study
		H. fuligineoviolaceum	simple-septa	simple-septa	Maas Geesteranus 1971 [26]
		H. fuscoindicum	simple-septa	simple-septa	Maas Geesteranus 1967 [65]
	Violaceum	H. glaucopus	simple-septa	simple-septa	Maas Geesteranus 1975 [1]
		H. joeides	simple-septa	simple-septa	Baird et al. 2013 [27]
		H. roseoviolaceum	simple-septa	simple-septa	Nitare et al. 2021 [53]

Group	Subgenus	Species	Pileus	Spines	References
I	Zonatum	H. atrorubrum H. bomiense H. concrescens H. dianthifolium H. parvum H. rubidofuscum H. scrobiculatum H. scrobiculatum H. subsuccosum H. subsuccosum H. sulcatum H. yunnanense Hydnellum sp 3 Hydnellum sp 5	simple-septa simple-septa simple-septa simple-septa simple-septa simple-septa simple-septa simple-septa simple-septa simple-septa simple-septa simple-septa simple-septa simple-septa	simple-septa simple-septa simple-septa simple-septa simple-septa simple-septa simple-septa simple-septa simple-septa simple-septa simple-septa simple-septa simple-septa simple-septa	In this study In this study Baird et al. 2013 [27] Loizides et al. 2016 [36] Baird et al. 2013 [27] In this study Baird et al. 2013 [27] In this study Baird et al. 2013 [27] In this study In this study
	Others	H. complicatum H. cumulatum H. lundellii H. martioflavum	simple-septa simple-septa simple-septa simple-septa	simple-septa simple-septa simple-septa simple-septa	Baird et al. 2013 [27] Baird et al. 2013 [27] Maas Geesteranus 1975 [1] Baird et al. 2013 [27]
П	Subindufibulatum	H. caeruleum H. ferrugipes	simple-septa, occasionally with clamp- connections simple-septa, occasionally	simple-septa simple-septa	Baird & Khan 1986 [2]; In this study Baird et al. 2013 [27]
		H. fibulatum	with clamp- connections simple-septa, occasionally with clamp- connections	simple-septa	In this study
III	Others	H. peckii	mostly with clamp- connections, minority of simple-septa mostly with	mostly with clamp- connections, minority of simple-septa	Baird et al. 2013 [27]; In this study
		H. versipelle	clamp- connections, minority of simple-septa	clamp- connections, minority of simple-septa	Baird et al. 2013 [27]
IV	Others	H. diabolus	clamp- connections	simple-septa	Baird et al. 2013 [27]
		H. atrospinosum	clamp- connections	clamp- connections	In this study
	Hydnellum	H. suaveolens	clamp- connections	clamp- connections	Baird et al. 2013 [27]
V	Continui	H. cyanopodium	clamp- connections	clamp- connections	Baird 1986 [51]
	Caesispinosum	H. scleropodium	clamp- connections	clamp- connections	Harrison 1964 [33]

Table 3. Cont.

Group	Subgenus	Species	Pileus	Spines	References
	Others	H. geogenium	clamp- connections	clamp- connections	Baird et al. 2013 [27]
		Sarcodon aspratus	clamp- connections	clamp- connections	Maas Geesteranus 1971 [26]
V Sarcodon	S. imbricatus	clamp- connections	clamp- connections	Baird et al. 2013 [27]	
		S. leucopus	clamp- connections	clamp- connections	Mleczko et al. 2011 [66]
	Sarcodon	S. quercinofibulatus	clamp- connections	clamp- connections	Pérez-De-Gregorio et al. 2011 [67]
		S. scabripes	clamp- connections	clamp- connections	Baird et al. 2013 [27]
		S. squamosus	clamp- clamp- connections connections		Baird 1986 [34]
	Others	H. regium	hyphae with fe and with a few c	ew simple-septa lamp-connections	Harrison 1964 [33]

Table 3. Cont.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/jof7100818/s1, Figure S1: Maximum likelihood tree illustrating the phylogeny of *Hydnellum* and *Sarcodon* based on ITS sequence dataset.

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Data Availability Statement: Publicly available datasets were analyzed in this study. All resulting alignments were deposited in TreeBASE (http://www.treebase.org (accessed on 17 August 2021); accession number S28676). All newly generated sequences were deposited in GenBank (https://www.ncbi.nlm.nih.gov/genbank/ (accessed on 23 September 2021); Table 1). All new taxa were deposited in Mycobank.

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